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DETECTION, MONITORING AND MANAGEMENT OF *RHIZOCTONIA SOLANI* AG 2-2 LP THAT CAUSES LARGE PATCH IN ZOYSIAGRASS IN TEXAS

A Dissertation

by

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Zoysiagrass (*Zoysia* Willd.) is among the most economically important warm-season turfgrasses produced and managed by the green industry. Its use is increasing throughout the southern United States due to superb characteristics and low requirement for cultural inputs. But a primary limitation is its susceptibility to large patch (LP), caused by the soil-borne fungus *Rhizoctonia solani* anastomosis group (AG) 2-2 LP. Knowledge is lacking concerning the effects of summertime cultural management and environmental conditions on *R. solani* AG 2-2 LP activity and LP symptom development in zoysiagrass in the fall and spring, particularly in Texas. Thus, thatch/soil moisture, thatch/soil temperature, air temperature, irrigation and nitrogen levels, and chemical control were among environmental and cultural variables evaluated in field and laboratory studies in conjunction with turfgrass quality, fungal activity and disease development. A toothpick baiting method developed to detect *Rhizoctonia* spp. in other crop systems was modified in this study for use in turfgrass. A semi-selective medium was also developed in this study to aid in isolating *R. solani* AG 2-2 LP. These studies demonstrated that thatch/soil moisture, thatch/soil temperature and air temperature are significant environmental factors affecting *R. solani* AG 2-2 LP activity. Irrigation levels correlated with turfgrass quality, thatch/soil moisture, thatch/soil temperature, fungal activity and disease development, but only during certain sampling/rating dates. Nitrogen levels correlated with turfgrass quality, thatch/soil temperature and disease development, but also only during certain sampling/rating dates. These studies also

demonstrated that turfgrass quality is acceptable and LP symptom development is minimized when zoysiagrass is irrigated at 36% to 60% of historical reference evapotranspiration and fertilized at an annual rate of 49 kg ha^{-1}. Chemical control of LP in zoysiagrass was inconsistent within and between study years. Fungicides varied in their residual activity, and differences in their effect on *R. solani* AG 2-2 LP activity and LP symptom development were significant only during certain sampling/rating dates. The toothpick baiting method, used in conjunction with monitoring of environmental conditions, can be a useful tool to predict *R. solani* AG 2-2 LP activity prior to LP symptom development and to determine the optimal time to apply fungicides preventatively.

DEDICATION

To my children and grandchildren ... I have made it a lifelong journey to take the road less traveled, and I have come again to another fork in the road, but the journey is not over. I believe it has made and will continue to make the difference between living an ordinary or extraordinary life. I hope that as you watched me pursue my dreams that I have been an inspiration for pursuing your own, and that I have been a model for self-discipline, steadfastness, fortitude, working hard and approaching everything with a spirit of excellence. The word "cannot" is not in my vocabulary, and I hope that it is not in yours either.

Last but not least, to my sunrise ... my sunset ... all the warm rays in between ... and the brightest moon in the darkest night ... after the dusk and before the dawn ... the journey is just beginning.

ACKNOWLEDGEMENTS

Sincere thanks and appreciation go to anyone who crossed my path during the three-plus years that it took to finish this fifth and final degree. Some of you provided only friendship and your ear, but you walked beside me and helped me stay the course as I explored yet another fork in the road. Now I am facing another, and sadly, it is time to part ways. But the memories will never leave me.

To my graduate adviser, Dr. Young-Ki Jo, thank you for the opportunity to continue this journey that was begun 10 years ago with the original goal of owning and operating a landscape management and design business. Today the goal is to conduct research, teach and mentor students of my own. My eyes have been opened to the fascinating fields of bioenvironmental science and plant pathology, and there is no turning back from the exploration that still waits.

Thank you to my committee members, Dr. Daniel Ebbole and Dr. Libo Shan, who also were among my instructors in the Department of Plant Pathology & Microbiology at Texas A&M University (TAMU), and to my other committee member, Dr. Benjamin Wherley, who is in the Department of Soil and Crop and Sciences at TAMU. Dr. Ebbole, I want you to know that I burned the proverbial midnight oil many a night for you. But it was because you expect great things from your students, and I expect great things from myself, so I did not want to let you or myself down. Dr. Shan, thank you for the opportunity to guest lecture twice in your absence for an Introduction to Bioenvironmental Science course. You also expanded my knowledge of plant-

microbe interactions by introducing me to the world of MAMPs, PAMPS, PTI, ETI and MAPKs, and the *Arabidopsis thaliana* model plant system. Dr. Wherley, unfortunately our interaction was limited to committee meetings and discussing results from our joint project at the TAMU Turfgrass Ecology Field Laboratory. But you come across as a gentle giant. You are the perfect example of patience and understanding.

Thank you to my other instructors in the Department of Plant Pathology & Microbiology at TAMU, Drs. Dennis Gross, Herman Scholtof, Mike Kolomiets, Brian Shaw, Marty Dickman and Joshua Yuan. I will only say this once. The all-nighters doing relentless homework and studying for tests was worth it, because my understanding of plant pathology skyrocketed here at TAMU.

Thank you to Tim Huber, superintendent at The Club at Carlton Woods in The Woodlands, TX, and his staff for allowing me to conduct two of my projects at the driving range. I always enjoyed our brief visits each month and sharing with you progress on the research. But I will not miss dodging golf balls. I will never forget the one that found its mark on the back of my thigh during my first visit to the course.

Thank you to the myriad of mates in the Jo lab. I especially want to thank Maxwell Handiseni. You and I began this journey together in 2011, and you and I will finish it together in 2015. You are at the top of a short list of those who helped me stay the course when the going got tough. I look forward to the transition from classmate to colleague. I also appreciate your assisting me with field work from time to time and with statistical analyses. Yoon Jeong, who worked in our lab the summer of 2014, also is thanked for assisting with statistical analyses. I want to thank William (Andrew)

Cromwell, who worked as our technician from January 2012 until August 2013, when he began his own journey as a graduate student at the TAMU College of Veterinary Medicine & Biomedical Sciences. Andrew often accompanied me to The Club at Carlton Woods and the TAMU Turfgrass Ecology Field Laboratory, and he put up with my obsessive expectation to record data accurately and neatly in a notebook. I hope I rubbed off onto you. Others that assisted me in the field include Joopil Yang, who was our visiting scientist from South Korea from August 2011 to August 2013, and Ruben Lopez, who was our technician from January to June 2014. Matt Zidek also assisted me in the field while he was an employee in the lab during the summer of 2013 after completing an undergraduate degree in bioenvironmental science at TAMU. He transitioned that fall to one of two master's degree students in the lab. Luis Moncayo is the other master's degree student. Luis, who hails from Ecuador, did not get the pleasure of assisting me with field or lab work. But he has been there for me as a friend and sometimes kept me company in the lab when he and I worked late. I have enjoyed watching Matt and Luis grow as researchers, and I am impressed by their diligence. I look forward to following their careers after they too graduate in 2015. Also assisting me in the field were Ruiheng "Cassie" Yin, Shan "Sophia" Zhao and Zheng Guo, who interned in the lab during the fall semesters in 2011, 2012 and 2013, respectively, in conjunction with a collaborative program at Michigan State University to provide undergraduate students from China with lab experience. Ruiheng is now a graduate student at Rutgers University. She and Sam Jeon are credited with developing the semi-selective medium that I used in this study. Sam was an undergraduate student at TAMU

and worked in the lab the first semester I was here. The newest member of the lab, Lin Yu started working during the fall semester of 2014 and assisted in molecular analyses of *Rhizoctonia* spp. isolates while I prepared my exit seminar and dissertation.

Last but not least, thank you to all the office staff in the Department of Plant Pathology & Microbiology at TAMU. You work behind the scenes to keep us graduate students on track administratively. But you also sometimes serve as parents and guidance counselors. And sometimes you feed us ... coffee and sweets miraculously appear in the mail room when we need a pick-me-up. What would we or the department do without you?

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CHAPTER I

INTRODUCTION

The green industry contributes nearly $15 billion annually to the Texas economy (Palma and Hall 2009), with sod production alone contributing approximately $178 million of that total (Falconer and Niemeyer 2006). Sod ranks as the ninth largest agricultural crop (Falconer and Niemeyer 2006), with production on more than 56,000 acres occurring in the eastern half of the state. St. Augustinegrass (*Stenotaphrum secundatum*), bermudagrass (*Cynodon dactylon*) and zoysiagrass (*Zoysia* Willd.) are among the most economically important warm-season turfgrass species produced and managed in Texas. Commercial and residential use of zoysiagrass is increasing due to the turfgrass' aesthetic appeal (medium to fine texture and rich green color), excellent heat and drought tolerance, good shade and cold tolerance, and low requirement for cultural inputs, such as fertilizer and pesticide (Beard 1972, Chandra et al. 2014, Duble 1989, Fry and Huang 2004, Fry et al. 2008, NTEP 2007, Okeyo et al. 2011, Patton and Reicher 2007, Wherley et al. 2011). However, knowledge is still lacking regarding best-management practices (BMPs), such as irrigation and nitrogen levels, needed to maximize the turfgrass' quality and performance during the growing season. Homeowners, and turfgrass producers and managers often use BMPs that were developed for St. Augustinegrass or bermudagrass. But cultural mismanagement of zoysiagrass in the summer can result in a decline in quality and performance that may make the turfgrass more susceptible to disease in the following fall and spring.

Turfgrass health plays a role in resistance, tolerance or susceptibility to disease. Zoysiagrass is very susceptible to large patch (LP) caused by *Rhizoctonia solani* (Green et al. 1993, Hyakumachi et al. 1998, Smiley et al. 2005). There is no zoysiagrass cultivar known to be resistant to the disease, which weakens the turfgrass and reduces its quality, and may kill it in a severe infection (Toda et al. 2004). LP often occurs during the fall and spring when turfgrass is entering or exiting winter dormancy, respectively, and when the weather is generally cool and wet (Emmons 2000, Green et al. 1993, Martinez et al. 2009). Disease symptoms in the fall include circular patches of blighted turfgrass, ranging in diameter from less than one meter to up to nearly eight meters (Green et al. 1993, Martinez et al. 2009, Tisserat et al. 1994). Leaf blades at the patch perimeter initially appear yellow, orange or reddish brown in color but then become tan and collapse to the ground, forming a mat (Green et al. 1993, Merrill 2011). Disease symptoms in the spring include thin, sunken light brown patches in turfgrass that are slower to break dormancy and may be invaded by weeds. Symptoms usually disappear and turfgrass recovers in the spring as the soil temperature increases. However, patches of diseased turfgrass tend to return and expand in the same location in the fall when environmental conditions are more favorable.

Proper diagnosis of disease and monitoring of the causal agent is imperative in developing economically sustainable BMPs that delay and/or suppress the development of symptoms. Monitoring and management of LP is made more difficult due to the biology of the causal agent (Agrios 2005). A basidiomycetous fungus that inhabits the soil and cannot be eliminated once established, *R. solani* rarely produces a spore in

nature (teleomorph *Thanatephorus cucumeris*) and only under special conditions in a laboratory. *R. solani* is a species complex comprised of many taxonomically related but genetically isolated groups (Anderson 1982, Carling et al. 2002, Gonzalez Garcia et al. 2006, Obasa 2012, Ogoshi 1987, Vilgalys and Cubeta 1994). *R. solani* isolates are identifiable at the species level through distinct mycelial characteristics, which include wide (4-15 μm) multinucleate hyphae that branch at right angles and a slight constriction and septum near each hyphal junction. Mycelia are colorless when young but turn yellowish, or light or dark brown with age. Below the species level, *R. solani* isolates have been assigned to an anastomosis group (AG) based on fusion of compatible hyphae. There are 14 AGs currently described in the literature (AG 1-13 and AG B1) (Carling et al. 2002). The concept of intraspecific groups (Ogoshi 1987) further differentiates isolates within AGs based on a combination of anastomosis, culture morphology, serological studies (Adams and Butler 1979), fatty acid analysis (Johnk and Jones 1993, Johnk et al. 1993), protein electrophoresis (Reynolds et al. 1983), nucleic acid studies, host range and pathogenicity (Grosch et al. 2006, Hyakumachi et al. 1998, Toda et al. 2004, White et al. 1990). Seven of the AGs (1, 2, 3, 4, 6, 8 and 9) have intraspecific groups (Carling et al. 2002, Ogoshi 1987). AG 2 is the most heterogeneous and has seven intraspecific groups (AG 2-1, AG 2-2 IIIB, AG 2-2 IV, AG 2-2 LP, AG 2-3, AG 2-4 and AG 2 B1) (Carling et al. 2002, Hyakumachi et al. 1998, Obasa 2012). AG 2-2 LP is the causal agent of large patch (Hyakumachi et al. 1998).

Isolation of *Rhizoctonia* spp. from soil can be difficult due to low inoculum density (Paulitz and Schroeder 2005). Thus, researchers developed methods using plant

tissue, including stems and seed (Papavizas and Davey 1962, Sneh et al. 1966), roots (LaMondia 1999, Paulitz et al. 2003) and whole plants (Gilligan et al. 1996), as bait to facilitate the study of fungal activity and spatial distribution. Kumar et al. (1999) took advantage of the fact *Rhizoctonia* spp. can decompose cellulose (Garrett 1962) and seems to grow faster along surfaces than through a porous substrate like sand (Otten and Gilligan 1998), and used wooden toothpicks as bait in sand in greenhouse assays to measure the linear spread of *R. solani* AG 8 and AG 11. Despite knowing *Rhizoctonia* spp. can decompose cellulose, Paulitz and Schroeder (2005) surmised that the interaction between the fungus and a toothpick is thigmotrophic rather than chemotrophic, and expected hyphae to attach and grow along the toothpick surface. And since baiting methods typically are not used to quantify *Rhizoctonia* spp. in a given space, Paulitz and Schroeder (2005) took the toothpick baiting method developed by Kumar et al. (1999) one step further, modifying it to facilitate both isolation and quantification of *R. solani* AG 8 and *R. oryzae* inside and outside of bare patches in wheat fields in eastern Washington. The researchers developed standard curves based on known amounts of inoculum added to natural soils and the volume of soil occupied by a toothpick. They then used those curves to predict inoculum density in the field, as determined by the number of hyphae colonizing toothpicks. Since isolation of *Rhizoctonia* spp. directly from soil and plant tissue can be difficult, the toothpick baiting method developed by Kumar et al. (1999) and Paulitz and Schroeder (2005) was further modified in this study to detect and monitor *R. solani* AG 2-2 LP activity in turfgrass. Since other soil-dwelling fungi and *Oomycetes* attach to toothpicks, a semi-selective medium also was developed

in this study to retard their growth and to enable microscopic identification and cultural isolation of *R. solani* AG 2-2 LP.

Management of LP is of particular concern to homeowners, and turfgrass producers and managers, who encounter economic loss due to blighting or dead turfgrass. LP is not curable, but symptoms may be delayed and/or suppressed by applying fungicides preventatively (Tisserat et al. 1994). When to treat for LP often is based on the calendar instead of changing environmental conditions, which often results in ineffective and costly fungicide applications. Environmental stressors, such as cold and drought, weaken turfgrass and can be factors in disease severity.

Knowledge is still lacking concerning specific environmental and cultural conditions that favor LP development in zoysiagrass and the optimal time to apply fungicides in Texas. Thus, thatch/soil moisture, thatch/soil temperature, air temperature, irrigation, nitrogen and the residual effect of chemical control were among conditions monitored in conjunction with *R. solani* AG 2-2 LP activity in Zeon® Zoysia [*Z. matrella* (L.) Merr.] (Bladerunner Farms, Inc., Poteet, TX) and 'Palisades' zoysiagrass (*Z. japonica* Steud.) (Texas A&M AgriLife Research, Dallas, TX) in field and laboratory assays. Data from assays were subjected to Analysis of Variance using the PROC GLM or REG procedure in SAS version 9.3 or 9.4 (SAS Institute Inc., Cary, NC). Means were separated using Fisher's Least Significant Difference test. Analyzed data then was used to develop BMPs to delay and/or suppress LP in zoysiagrass in Texas.

CHAPTER II

SEASONAL DYNAMICS OF *RHIZOCTONIA SOLANI* AG 2-2 LP IN ZOYSIAGRASS IN FIELD AND LABORATORY STUDIES

INTRODUCTION

Rhizoctonia solani causes a patch disease in both cool- and warm-season turfgrasses (Green et al. 1993, Piper and Coe 1919). But what was once considered the same disease in both types of turfgrass now is not, and disease is differentiated not only by the type of turfgrass but also by the cultural type of the soil-borne necrotrophic fungus that causes symptoms. Proper diagnosis and management of patch diseases is made more difficult by the fact that *R. solani* is a species complex comprised of many taxonomically related but genetically isolated groups (Anderson 1982, Carling et al. 2002, Gonzalez Garcia et al. 2006, Obasa 2012, Ogoshi 1987, Vilgalys and Cubeta 1994). Isolates traditionally have been assigned to an anastomosis group (AG) based on fusion of compatible hyphae. There are 14 AGs currently described in the literature (AG 1-13 and AG B1) (Carling et al. 2002). AG 1, 2, 3, 4, 6, 8 and 9 were further differentiated into intraspecific groups (Carling et al. 2002, Ogoshi 1987), based also on culture morphology, pathogenicity, serological studies (Adams and Butler 1979), fatty acid analysis (Johnk and Jones 1993, Johnk et al. 1993), protein electrophoresis (Reynolds et al. 1983), nucleic acid studies and other criteria. AG 2 is the most heterogeneous of the AGs. It is differentiated into seven intraspecific groups (2-1, 2-2 IIIB, 2-2 IV, 2-2 LP, 2-3, 2-4 and B1) (Carling et al. 2002, Hyakumachi et al. 1998,

Obasa 2012) and is known to form bridging anastomosis reactions with AG 3 and AG 8 (Carling 1996, Carling et al. 2002, Obasa 2012).

Methods used to differentiate intraspecific groups determined that *R. solani* AG 1 and *R. solani* AG 2-2 IIIB are the causal agents of brown patch in cool-season turfgrasses (Burpee and Martin 1992, Piper and Coe 1919, Smiley et al. 1992), such as bentgrass (*Agrostis* spp.), bluegrass (*Poa* spp.), fescue (*Festuca* spp.) and ryegrass (*Lolium* spp.). *R. solani* AG 2-2 LP is the causal agent of large patch (LP) in warm-season turfgrasses (Green et al. 1993, Hyakumachi et al. 1998, Smiley et al. 2005), such as bermudagrass (*Cynodon dactylon*), centipedegrass (*Eremochloa ophiuroides*), St. Augustinegrass (*Stenotaphrum secundatum*) and zoysiagrass (*Zoysia* Willd.). Both patch diseases also can be differentiated based on their epidemiology. Brown patch commonly occurs during the summer, while LP commonly occurs during the fall and spring. Various cultural and environmental factors are known to contribute to development and severity of disease. Both patch diseases are not curable, due to the biology of *R. solani*. Once established in the soil, the fungus cannot be eliminated. But disease development may be delayed and/or symptom severity suppressed by utilizing less susceptible turfgrass cultivars and by practicing a combination of cultural and chemical management strategies, including the application of fungicides preventatively prior to the onset of symptoms (Tisserat et al. 1994). But when to treat for LP often is based on the calendar instead of changing environmental conditions, which often results in ineffective and costly fungicide applications.

The green industry contributes nearly $15 billion annually to the Texas economy (Palma and Hall 2009), with sod production alone contributing approximately $178 million of that total (Falconer and Niemeyer 2006). Sod ranks as the ninth largest agricultural crop (Falconer and Niemeyer 2006), with production on more than 56,000 acres occurring in the eastern half of the state. Zoysiagrass is among the most economically important turfgrasses produced and managed in Texas. Commercial and residential use is increasing due to the turfgrass' aesthetic appeal (medium to fine texture and rich green color), excellent heat and drought tolerance, good shade and cold tolerance, and low requirement for cultural inputs, such as fertilizer and pesticide (Beard 1972, Chandra et al. 2014, Duble 1989, Fry and Huang 2004, Fry et al. 2008, NTEP 2007, Okeyo et al. 2011, Patton and Reicher 2007, Wherley et al. 2011). But despite its desirable attributes, zoysiagrass is very susceptible to LP (Smiley et al. 2005), and there are no cultivars known to be resistant to the disease. LP is of particular concern to turfgrass producers and managers, who encounter significant economic loss as a result of chemical inputs to avoid marketing sod at a lower value due to blighting. Thus, there is a need for better cultural and chemical management practices, and especially for those practices that would provide an economic benefit to turfgrass producers and managers.

LP is a serious disease that weakens turfgrass and reduces its quality, and kills it in a severe infection (Toda et al. 2004). LP often occurs when turfgrass is entering or exiting winter dormancy in the fall and spring, respectively, and when the weather is generally cool and wet (Emmons 2000, Green et al. 1993, Martinez et al. 2009). Actively growing turfgrass tends not to exhibit disease symptoms unless environmental

conditions, such as moisture and temperature, are favorable. It is known that disease symptoms can occur when the temperature of the thatch layer is between 10°C and 21°C, and when leaf blade wetness is continuous for at least 48 hours (Tredway and Burpee 2001).

Symptoms of LP in the fall include circular patches of blighted turfgrass, ranging in diameter from less than one meter to up to nearly eight meters (Green et al. 1993, Martinez et al. 2009, Tisserat et al. 1994) (Figure 2.1). Leaf blades at the patch perimeter initially appear yellow, orange or reddish brown in color but then become tan and collapse to the ground, forming a mat (Green et al. 1993, Merrill 2011). Symptoms of LP in the spring include thin, sunken light brown patches in turfgrass that are slower to break dormancy and may be invaded by weeds (Figure 2.1). Symptoms usually disappear and turfgrass recovers in the spring as the soil temperature increases. However, patches of diseased turfgrass tend to return and expand in the same location in the fall when environmental conditions are more favorable.

Knowledge is still lacking concerning specific environmental conditions in Texas that contribute to development and severity of LP in zoysiagrass and concerning the optimal time to apply fungicides to delay and/or suppress disease. Environmental conditions are not always uniform in a particular location. And on a lawn, or a golf course fairway or putting green, where the turfgrass is genetically uniform, one would expect *R. solani* AG 2-2 LP isolates collected from diseased turfgrass to be clonal and genetically similar, if not identical, from patch to patch. But that is not always the case. Whereas isolates collected from the same patch have been reported to be genetically

similar, isolates collected from a different patch in the same location have been reported to be different. Thus, monitoring of fungal activity and proper diagnosis of disease is imperative in developing economically sustainable best-management practices (BMPs) that delay and/or suppress LP in Texas.

Figure 2.1. Symptoms in zoysiagrass in the fall (A and B) and spring (C and D) of large patch caused by *Rhizoctonia solani* AG 2-2 LP. Symptoms in the fall include circular patches of blighted turfgrass, ranging in diameter from less than one meter to nearly eight meters. Leaf blades at the patch perimeter initially appear yellow, orange or reddish brown in color but then become tan and collapse to the ground, forming a mat. Symptoms in the spring include thin, sunken light brown patches in the turfgrass that are slower to break dormancy and may be invaded by weeds.

Proper diagnosis of disease requires isolation of the causal agent. Isolation of *Rhizoctonia* spp. from soil can be difficult due to low inoculum density (Paulitz and Schroeder 2005). Thus, researchers have developed methods using plant tissue, including stems and seed (Papavizas and Davey 1962, Sneh et al. 1966), roots (LaMondia 1999, Paulitz et al. 2003) and whole plants (Gilligan et al. 1996), as bait to facilitate the study of fungal activity and spatial distribution. Kumar et al. (1999) took advantage of the fact *Rhizoctonia* spp. can decompose cellulose (Garrett 1962) and seems to grow faster along surfaces than through a porous substrate like sand (Otten and Gilligan 1998), and used wooden toothpicks as bait in sand in greenhouse assays to measure the linear spread of *R. solani* AG 8 and AG 11. Despite knowing *Rhizoctonia* spp. can decompose cellulose, Paulitz and Schroeder (2005) surmised that the interaction between the fungus and a toothpick is thigmotrophic rather than chemotrophic, and expected hyphae to attach and grow along the toothpick surface. And since baiting methods typically are not used to quantify *Rhizoctonia* spp. in a given space, Paulitz and Schroeder (2005) took the toothpick baiting method developed by Kumar et al. (1999) one step further, modifying it to facilitate both isolation and quantification of *R. solani* AG 8 and *R. oryzae* inside and outside of bare patches in wheat fields in eastern Washington. The researchers developed standard curves based on known amounts of inoculum added to natural soils and the volume of soil occupied by a toothpick. They then used those curves to predict inoculum density in the field, as determined by the number of hyphae colonizing toothpicks.

Objectives of this study were to adapt the toothpick baiting method for use in turfgrass in field and laboratory assays, and to evaluate *R. solani* AG 2-2 LP activity in zoysiagrass in Texas, as influenced by thatch/soil moisture and air temperature. Since other soil-dwelling fungi and *Oomycetes* also attach to toothpicks, a semi-selective medium was developed in this study to retard their growth and to enable microscopic identification and isolation of *R. solani* AG 2-2 LP. Isolates were confirmed based on cultural morphology and genomic DNA analyses using conventional polymerase chain reaction (PCR) and primer pairs universal for fungal species and specific for LP. Environmental and *R. solani* AG 2-2 LP activity data were subjected to statistical analyses to determine seasonal relationships.

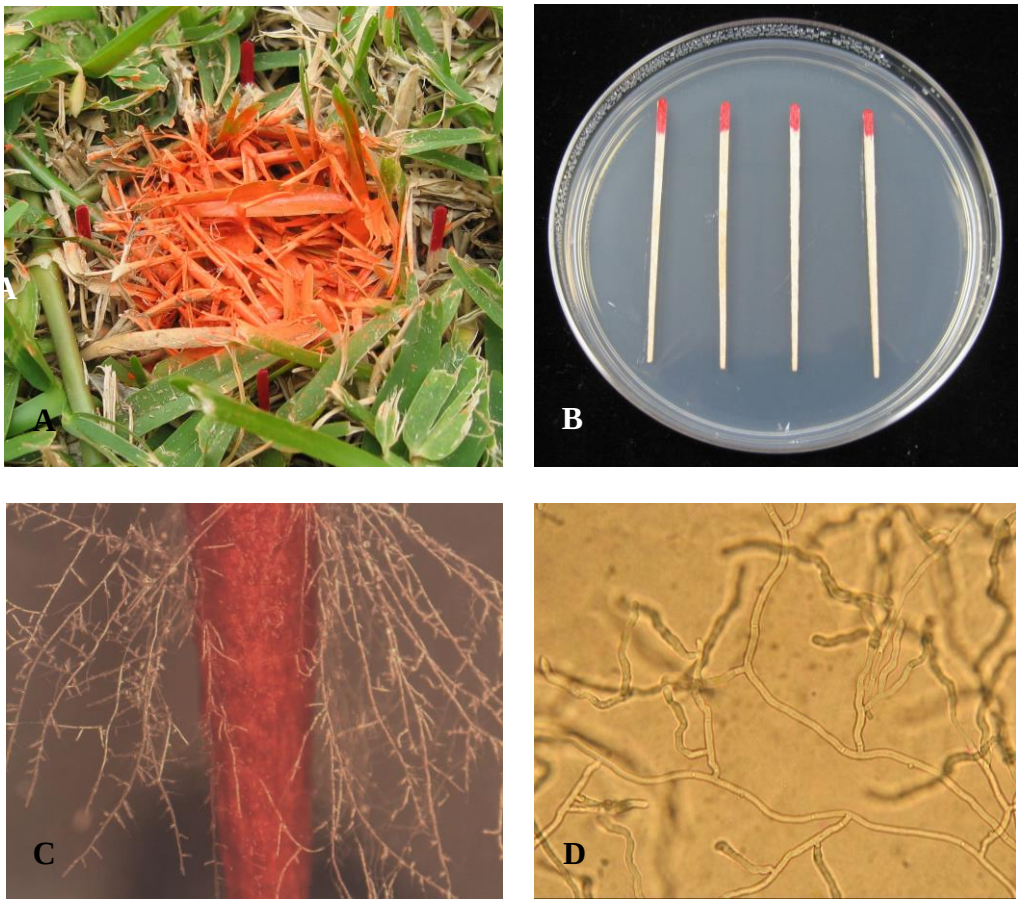
MATERIALS AND METHODS

Thatch/soil moisture and air temperature were monitored in conjunction with monthly sampling to detect *R. solani* AG 2-2 LP during a two-year field study conducted from October 2011 to September 2013 at a driving range at The Club at Carlton Woods in The Woodlands, TX. The driving range is planted in Zeon® Zoysia [*Z. matrella* (L.) Merr.] (Bladerunner Farms, Inc., Poteet, TX), contained naturally occurring populations of *Rhizoctonia* spp. and was not treated for LP with any fungicides. Seasonal temperatures associated only with *R. solani* AG 2-2 LP activity were then used as treatments in a growth chamber study to evaluate field data and to monitor fungal activity in growing medium and Zeon® zoysiagrass sod plugs that were inoculated with an isolate of *R. solani* AG 2-2 LP.

Driving range study. *Detection of R. solani AG 2-2 LP.* Fluorescent paint was used to randomly mark sampling spots (~5 cm in diameter) in the turfgrass. The number of spots varied each month. Spots were sampled for *R. solani* AG 2-2 LP using four flat-sided wooden toothpicks (Jarden Home Brands, Daleville, IN) that were colored on the rounded end (~6 mm) with a red permanent marker and then inserted into the turfgrass in a cross pattern, just outside each spot and up to the bottom of the red mark (Figure 2.2). Toothpicks were removed after 48 h and plated onto 1.5% water agar (WA) medium amended with 100 mg/L chloramphenicol (CalBiochem/EMD Biosciences, Inc., La Jolla, CA), 1000 mg/L mefenoxim (Quali-Pro®, Raleigh, NC) and 5 µg/L propiconazole (Quali-Pro®, Raleigh, NC) (Figure 2.2) (Appendix A). Plates were incubated 18 to 24 h at 25°C in the dark, and then toothpicks were examined microscopically for attachment of hyphae characteristic of *R. solani* AG 2-2 LP (Hyakumachi et al. 1998) (Figure 2.2).

Monitoring of thatch/soil moisture and air temperature. Volumetric water content in the top 7 cm of thatch/soil in the turfgrass was measured using a TH300 Big Stick Soil Moisture Probe (Dynamax Inc., Houston, TX). Two measurements were taken and averaged to derive a mean percentage of thatch/soil moisture for each sampling spot during each 48-h sampling period. Measurements were taken when toothpicks were inserted and when they were removed from the turfgrass. Low and high air temperatures for The Woodlands, TX, during each 48-h sampling period were retrieved from an online database at the Weather Underground (http://www.wunderground.com) and AccuWeather (http://www.accuweather.com) and averaged to derive a mean daily air temperature.

Figure 2.2. Toothpick baiting method illustrated. A) Toothpicks inserted into turfgrass for 48 h and then B) plated onto semi-selective medium. C) Toothpicks observed microscopically 18 to 24 h after incubation at 25°C in the dark for hyphae characteristic of *Rhizoctonia solani* AG 2-2 LP. D) *R. solani* AG 2-2 LP colony that grew from hyphal tip transferred to 2.0% water agar medium and incubated 2 to 4 d at 25°C in the dark.



Statistical analyses. Spots sampled each month were scored for fungal activity based on the absence (0) or presence (1) on at least one of the four toothpicks of hyphae characteristic of *R. solani* AG 2-2 LP. To determine the seasonal effects of thatch/soil moisture and air temperature (independent variables) on *R. solani* AG 2-2 LP activity (dependent variable), data for the sampling periods from November to March, when the fungus was speculated to be active, were subjected to regression analysis between

dependent and independent variables using the REG procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed with respect to year (2011-2012 or 2012-2013) as well as the study as a whole (2011-2013). SAS code used in analyses is shown in Appendix B.

Growth chamber study. *Rhizoctonia spp. isolates used in this study.* The toothpick baiting method previously described in the driving range study was used to obtain *R. solani* AG 2-2 LP isolates CW570A and CW25 in November 2011 and February 2013, respectively, from the driving range at The Club at Carlton Woods in The Woodlands, TX. The method was also used to obtain *R. solani* AG 2-2 LP isolates TAM-Ja-26, TAM-F-2-1 and TAM-M-4-31 in January 2013, February 2013 and March 2014, respectively, from an established stand of 'Palisades' zoysiagrass (*Z. japonica* Steud.) (Texas A&M AgriLife Research, Dallas, TX) at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. *R. zeae* isolates TAM-J-2-25 and TAM-J-4-12 were obtained in June 2012 from the same stand of 'Palisades' zoysiagrass. *Rhizoctonia* spp. isolates were identified by cultural morphology (Figure 2.3) and by conventional PCR analyses of genomic DNA (Figure 2.4) using universal primer pair ITS1-ITS4 (White et al. 1990) that amplifies the internal transcribed spacer (ITS) region in fungi and using specific primer pair A091F-A091R (Toda et al. 2004) that differentiates *R. solani* AG 2-2 LP isolates below the species level.

Figure 2.3. A) *Rhizoctonia solani* AG 2-2 LP and B) *R. zeae* isolates grown on potato dextrose agar medium that were identified by cultural morphology in this study.

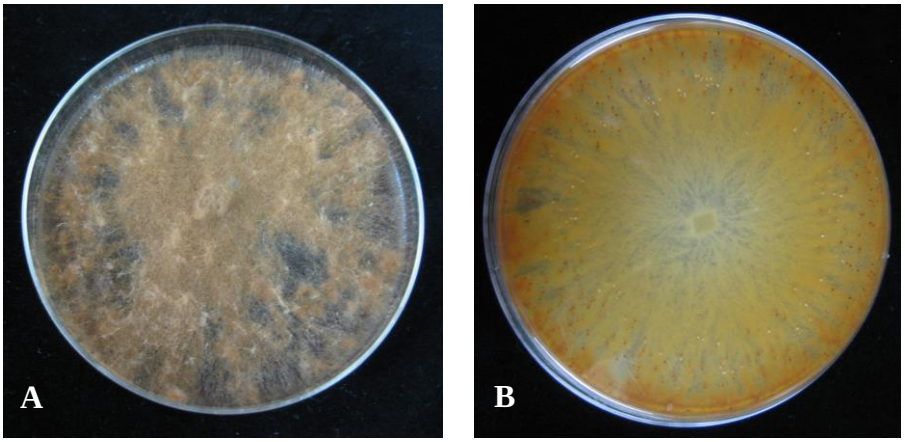
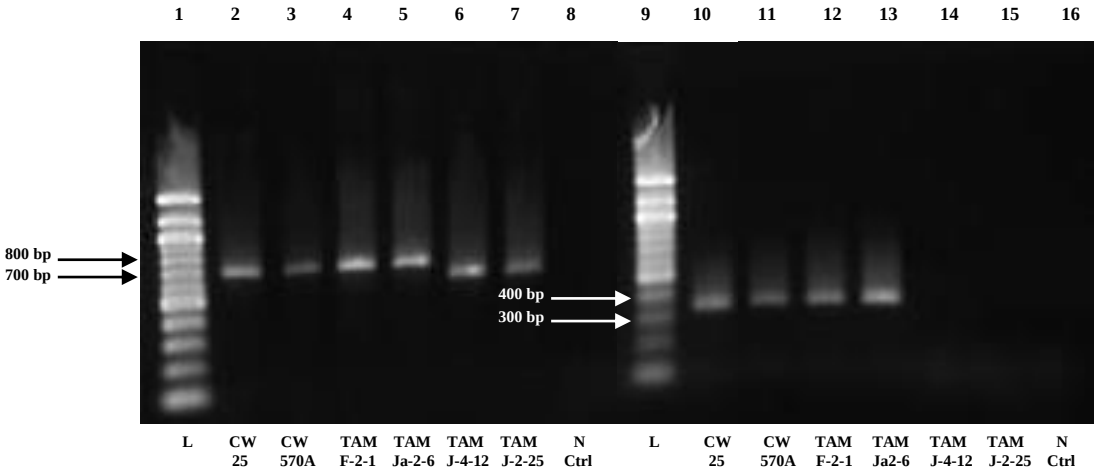


Figure 2.4. *Rhizoctonia solani* and *R. zeae* isolates identified using conventional polymerase chain reaction and universal primer pair ITS1-ITS4 that amplifies the nuclear small-subunit ribosomal DNA sequence in fungi and specific primer pair A091F-A091R that differentiates *R. solani* AG 2-2 LP below the species level. The ITS1-ITS4 primer pair produced an expected 740-base-pair (bp) amplicon from genomic DNA of *R. solani* isolates (lanes 2-5). The A091F-A091R primer pair produced an expected 350-bp amplicon from genomic DNA of *R. solani* AG 2-2 LP isolates (lanes 10-13). Negative controls consisted of master mix and water instead of a DNA template (lanes 8 and 16). Size of amplicons was determined by comparison to a 100-bp DNA ladder (L).



Preparation of genomic DNA. A hyphal tip of *R. solani* AG 2-2 LP isolate TAM-M-4-31 was transferred from semi-selective medium to 2.0% WA medium and incubated 2 to 4 d at 25°C in the dark. A hyphal tip that grew out of the plug (Figure 2.2) was transferred to potato dextrose agar (PDA) medium and incubated 7 to 10 d at 25°C in the dark. Genomic DNA was isolated from the pure culture using a method developed by Saitoh et al. (2006) that was modified in this study (Appendix C). Long-term storage was at -20°C.

PCR protocols. Genomic DNA was quantified, using the NanoDrop 1000 version 3.8.1 spectrophotometer (NanoDrop Technologies, Wilmington, DE), prior to conventional PCR analyses. PCR samples using the ITS1-ITS4 primer pair were prepared using the GoTaq® PCR Core System I (Promega Corporation, Madison, WI) and contained 5X Colorless GoTaq® Flexi Buffer (Mg-free), 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 20 pmol of each primer, 20-200 ng/µl genomic DNA, 0.625 U GoTaq® DNA Polymerase and sterile ddH_20, for a total reaction volume of 25 µl. PCR samples using the A091F-A091R primer pair were prepared using the GoTaq® PCR Core System I (Promega Corporation, Madison, WI) and contained 5X Colorless GoTaq® Flexi Buffer (Mg-free), 1.5 mM $MgCl_2$, 0.3 mM of each dNTP, 25 pmol of each primer, 20-200 ng/µl genomic DNA, 0.625 U GoTaq® DNA Polymerase and sterile ddH_20, for a total reaction volume of 25 µl. Amplification was conducted using an Applied Biosystems 2720 Thermal Cycler (Life Technologies Corporation, Carlsbad, CA). The thermocycler program for the ITS1-ITS4 primer pair was as follows: initial denaturation of 3 min at 95°C; 35 cycles of 45 sec at 95°C, 45 sec at 50°C, and 1 min at 72°C; and a

final extension of 5 min at 72°C. The thermocycler program for the A091F-A091R primer pair was as follows: initial denaturation of 3 min at 94°C; 40 cycles of 1 min at 94°C, 1 min at 65°C, and 3 min at 72°C; and a final extension of 7 min at 72°C. Amplicons were resolved by electrophoresis in a 1% agarose gel in 1X TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0). The gel was post-stained 30-45 min in 1X TAE buffer containing 50 μg/ml of ethidium bromide and then washed 5 min in 1X TAE buffer. PCR products were then visualized using an AlphaImager® HP version 3.4.0.0 image acquisition and analysis system (ProteinSimple, San Jose, CA). The size of amplicons was determined by comparison to a 100-base pair (bp) ladder (New England BioLabs Inc., Ipswich, MA).

Collection of zoysiagrass sod plugs. A cup cutter was used to collect Zeon® zoysiagrass sod plugs (10 cm x 10 cm) from a field nursery at The Club at Carlton Woods in The Woodlands, TX, in mid-April 2014. Textural analysis indicates the soil is comprised of 92% sand, 6% silt and 2% clay (Tournament Turf Laboratories, Inc., Valencia, PA), and has an organic content of 13-15% (Thomas Turf Services, Inc., College Station, TX). The soil is slightly alkaline at a pH of 7.1, and the Cation Exchange Capacity is low at 2.86 (Tournament Turf Laboratories, Inc., Valencia, PA). Plugs were placed into zip lock plastic bags and transported in a cooler to the Department of Plant Pathology & Microbiology at Texas A&M University in College Station, TX. Bottoms of plugs were trimmed with a serrated kitchen knife and washed in tap water to remove the sand and clay soil layers but retain the root system. Plugs were then transplanted into 15-cm Kord Regal® standard nursery pots (Myers Industries Lawn

and Garden Group, Twinsburg, OH) that had been filled with 946.4 cubic centimeters of Sunshine® Professional Growing Mix (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) containing 70% to 80% Canadian sphagnum peat moss, horticultural grade perlite and dolomite limestone. The growing medium was rehydrated with tap water prior to transplanting. Pots were maintained in a greenhouse at Texas A&M University in College Station, TX, until early June 2014 when the turfgrass was used in this study. Pots were watered to saturation with tap water two to three times a week, and the turfgrass was trimmed level to the top of the pot once a week using scissors that had been sterilized with 70% EtOH. The turfgrass also was fertilized one week after transplanting and then every two weeks thereafter at the lowest labeled rate (1 teaspoon in 1 gallon of water) with an all-purpose fertilizer (Scotts Miracle-Gro Products, Inc., Marysville, OH) containing 24% total nitrogen (3.5% ammoniacal and 20.5% urea), 8% phosphorus (diphosphorus pentoxide) and 16% potassium (potassium oxide). The last fertilization occurred one week prior to using the turfgrass in this study. Also prior to use, the turfgrass was checked for natural infection by *R. solani* AG 2-2 LP using the toothpick baiting method.

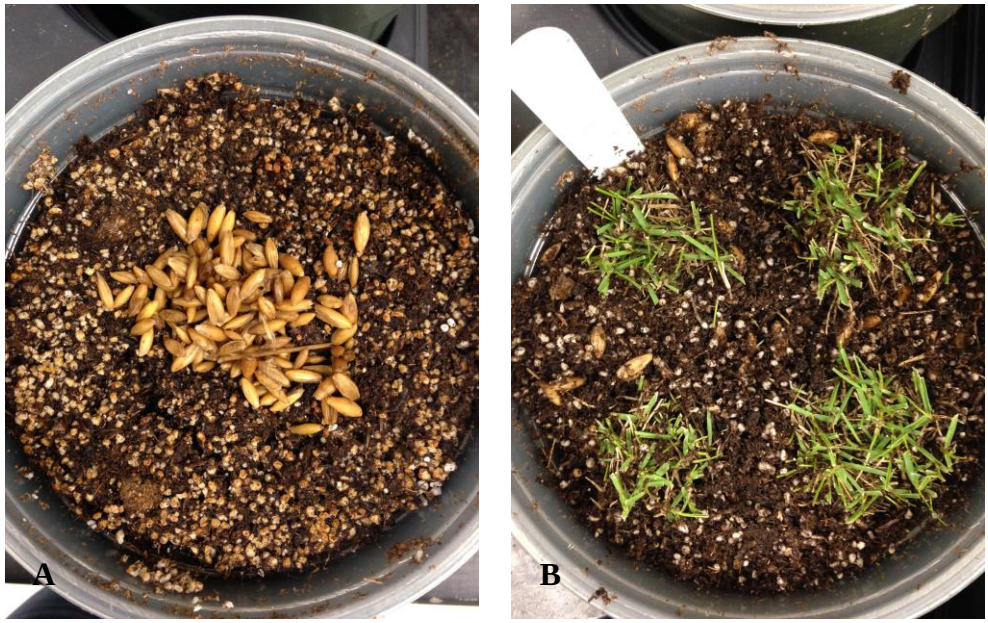
Preparation of inoculum. Using a method developed by Tisserat et al. (1989) that was slightly modified in this study, 250 ml of whole oat kernels were mixed with 500 ml of deionized water in a 4-L Erlenmeyer flask and sterilized by autoclaving twice, 24 h apart, for 30 min at 121°C. Plugs from an entire culture of *R. solani* AG 2-2 LP isolate TAM-M-4-31, grown 7 d on PDA in the dark, were mixed in the flask with the sterilized kernels, which had been cooled to ambient temperature, and then the flask was incubated

at 25°C in the dark. The flask was shaken periodically during incubation to ensure even distribution of fungal mycelia. Infestation was checked after 14 d by plating several kernels on PDA and then microscopically examining them 2 to 3 d after incubation at 25°C in the dark.

Inoculation of growing medium and zoysiagrass sod plugs. Twenty-four 15-cm Kord Regal® standard nursery pots (Myers Industries Lawn and Garden Group, Twinsburg, OH) were filled with 1,182.9 cubic centimeters of the Sunshine® Professional Growing Mix, and the growing medium was rehydrated with tap water. Twelve of the pots were then inoculated with 10g of the infested oat kernels by mixing the kernels into the upper 5 cm of potting medium (Figure 2.5). The remaining 12 pots were not inoculated and served as negative controls. The Zeon® zoysiagrass sod plugs being maintained in a greenhouse at Texas A&M University were cut into smaller plugs (2.5 cm x 2.5 cm x 5 cm) using a serrated kitchen knife. Four of these smaller plugs were transplanted, in a square pattern, into each of the 24 pots (Figure 2.5). A randomized complete block design, with 12 replications of six temperature treatments, was used to arrange inoculated and control pots. Three blocks were created for each set of 12 pots by randomly selecting and placing four pots into an aluminum foil baking pan. For the purpose of data collection, pots in each pan were labeled according to block and number in that block (inoculated pots: 1-1, 1-2, 1-3, 1-4, 2-1, 2-2, 2-3, 2-4, 3-1, 3-2, 3-3, 3-4; control pots: C1-1, C1-2, C1-3, C1-4, C2-1, C2-2, C2-3, C2-4, C3-1, C3-2, C3-3). All pots were simultaneously incubated in a VWR® Signature Diurnal Growth Chamber, Model 2015 2015-2 (Sheldon Manufacturing, Inc., Cornelius, OR) for two weeks at

25°C and under a 16-h photoperiod to optimize both the growth of the fungus and the growth of the sod plugs (Gelernter and Stowell 2005, Glenn 2013, Hyakumachi et al. 1998, Woods 2013). Throughout incubation, location of trays was maintained, and pots were watered to saturation every 3 to 5 d with tap water. Mean volumetric water content in the top 7 cm of only growing medium in the center of each pot was monitored as previously described for thatch/soil in the driving range study. Inoculation was checked twice by the toothpick baiting method, with a slight modification, beginning 6 and 10 d after incubation. In all pots, one toothpick was inserted into the center of each of the four sod plugs, and one toothpick was inserted into the center of the space between each sod plug that contained only growing medium. Control pots were similarly checked for any natural infection by *R. solani* AG 2-2 LP.

Figure 2.5. A) Inoculation of growing medium with whole oat kernels infested with an isolate of *Rhizoctonia solani* AG 2-2 LP prior to B) planting Zeon® zoysiagrass sod plugs.



Temperature treatments. During this study that began June 18, 2014, and ended November 11, 2014, inoculated and control pots were subjected twice to a series of decreasing and then increasing temperatures that simulate changing seasonal conditions in the field from fall (20°C, 15°C, 10°C) through spring (15°C, 20°C, 25°C). To offset an expected reduction in growth potential of the sod plugs due to the fluctuation in temperature, and to focus only on the effect of temperature on fungal activity, the 16-h photoperiod was maintained throughout the study. Location in the incubator and watering of pots were as described for the initial incubation after inoculation with infested oat kernels. Duration of each temperature treatment was 12 d. Sampling for *R. solani* AG 2-2 LP by the toothpick baiting method in sod plugs and growing medium was initiated 10 d after incubation at each temperature. Changes in temperature coincided with the pulling and plating of toothpicks 12 d after incubation at each temperature.

Assessment of temperature treatments. Fungal activity in sod plugs and growing medium in each pot was quantified separately based on the mean percentage of area on toothpicks (four in sod plugs and four in growing medium) that hyphae attached and the mean density of hyphae. Percentage of area was determined by measuring in centimeters the total area on a toothpick containing hyphae, dividing the total by the approximate length of a toothpick (7 cm) and multiplying by 100. Density was determined by a scale developed in this study, whereby 0 = no hyphae present; 1 = hyphae present and countable; 2 = hyphae overlapping but somewhat countable; and 3 = hyphae overlapping and no longer countable.

Statistical analyses. Data on attachment and density of hyphae on toothpicks inserted into sod plugs and growing medium were subjected to ANOVA using the PROC GLM procedure in SAS version 9.3. Means of significant independent variables were separated using Fisher's protected least significant difference (LSD) test at $P = 0.05$. Data were analyzed with respect to series of temperature treatments as well as the study as a whole. SAS code used in analyses is shown in Appendix D.

RESULTS

Driving range study. *R. solani AG 2-2 LP activity in relation to thatch/soil moisture.* The effect of thatch/soil moisture on *R. solani* AG 2-2 LP activity at the driving range at The Club at Carlton Woods was significant the first year ($P = 0.0037$) and second year ($P < 0.0001$). Fungal activity the first year was observed when the mean percentage of thatch/soil moisture was between 35.6% and 53.3% (Figure 2.6) (Table 2.1). Fungal activity the second year was observed when the mean percentage of thatch/soil moisture was between 28.3% and 43.9% (Figure 2.7) (Table 2.2).

R. solani AG 2-2 LP activity in relation to air temperature. The effect of air temperature on *R. solani* AG 2-2 LP activity at the driving range at The Club at Carlton Woods was significant the first year ($P < 0.0001$) but not the second year ($P = 0.1691$). Fungal activity the first year was observed when the mean daily air temperature was between 9.2°C and 20.8°C (Figure 2.8) (Table 2.1). Fungal activity the second year was observed when the mean daily air temperature was between 5.7°C and 11.7°C (Figure 2.9) (Table 2.2).

Figure 2.6. The effect of thatch/soil moisture on *Rhizoctonia solani* AG 2-2 LP activity in Zeon® zoysiagrass per 48-h sampling period each month from November 2011 to March 2012 at a driving range at The Club at Carlton Woods in The Woodlands, TX. The blue bars denote detection. The red trend line denotes thatch/soil moisture.

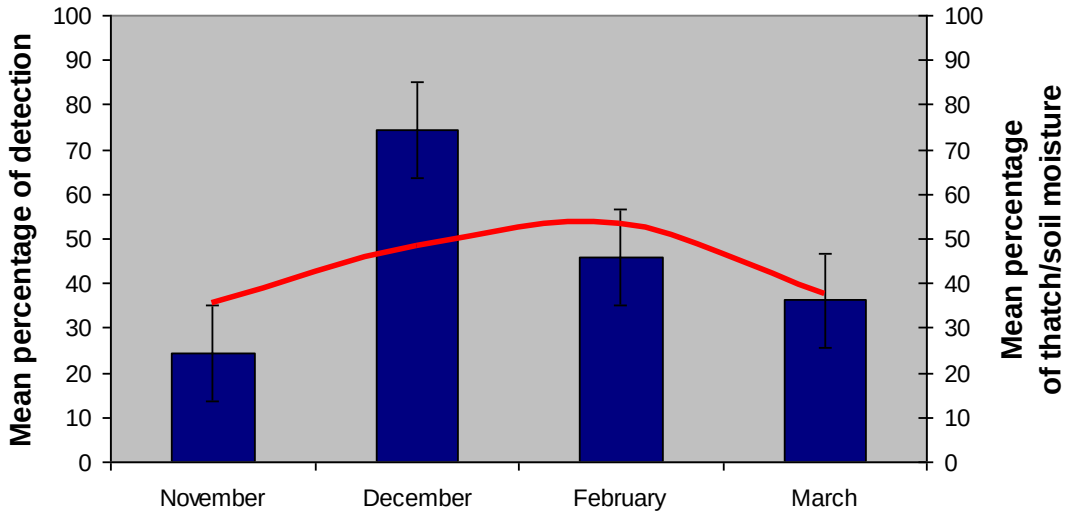


Table 2.1. Mean percentage of detection of *Rhizoctonia solani* AG 2-2 LP in Zeon® zoysiagrass in relation to mean percentage of thatch/soil moisture and mean daily air temperature per 48-h sampling period each month from November 2011 to March 2012 at a driving range at The Club at Carlton Woods in The Woodlands, TX. NR = data not recorded.

Month	Sampling spots	Mean % detection	Mean % thatch/soil moisture	Mean daily air temperature (°C)
November	74	24.3	35.6	20.2
December	82	74.4	48.4	9.2
January	50	36.0	NR	9.5
February	50	46.0	53.3	20.4
March	47	36.2	37.5	20.8

Figure 2.7. The effect of thatch/soil moisture on *Rhizoctonia solani* AG 2-2 LP activity in Zeon® zoysiagrass per 48-h sampling period each month from November 2012 to March 2013 at a driving range at The Club at Carlton Woods in The Woodlands, TX. The blue bars denote detection. The red trend line denotes thatch/soil moisture.

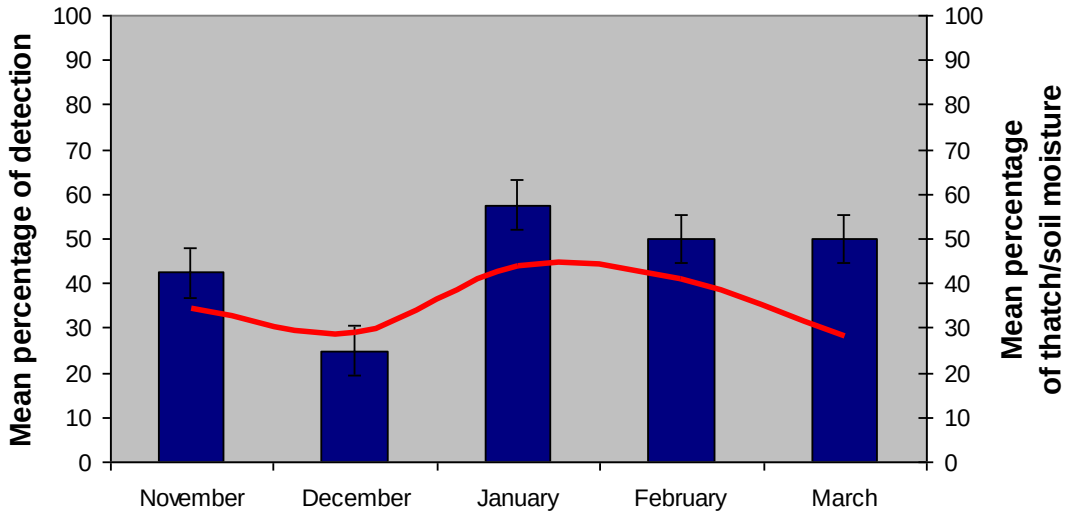


Table 2.2. Mean percentage of detection of *Rhizoctonia solani* AG 2-2 LP in Zeon® zoysiagrass in relation to mean percentage of thatch/soil moisture and mean daily air temperature per 48-h sampling period each month from November 2012 to March 2013 at a driving range at The Club at Carlton Woods in The Woodlands, TX.

Month	Sampling spots	Mean % detection	Mean % thatch/soil moisture	Mean daily air temperature (°C)
November	40	42.5	34.1	11.7
December	40	25.0	29.1	5.7
January	40	57.5	43.9	14.3
February	40	50.0	40.9	9.6
March	40	50.0	28.3	10.7

Figure 2.8. The effect of air temperature on detection of *Rhizoctonia solani* AG 2-2 LP in Zeon® zoysiagrass per 48-h sampling period each month from November 2011 to March 2012 at a driving range at The Club at Carlton Woods in The Woodlands, TX. The blue bars denote detection. The yellow trend line denotes air temperature.

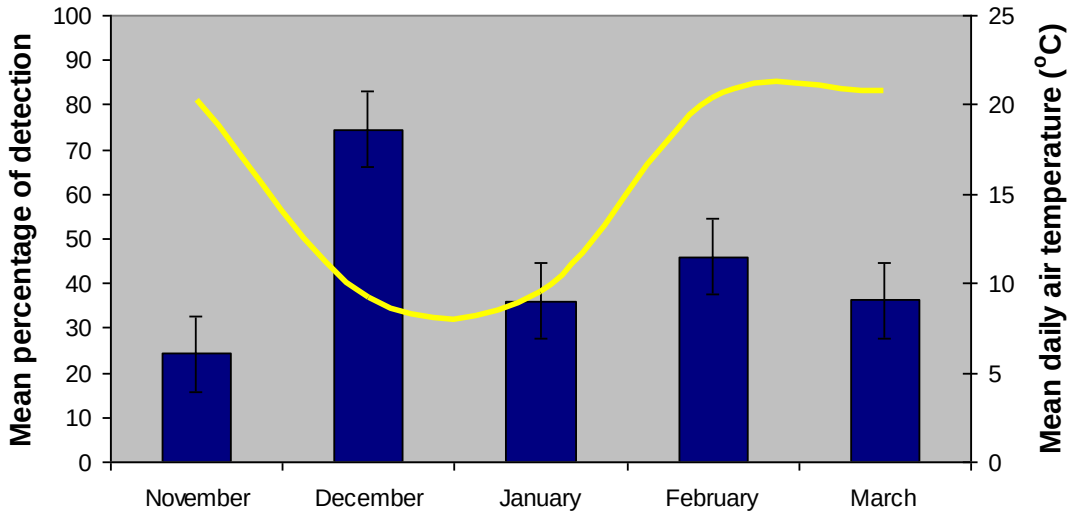
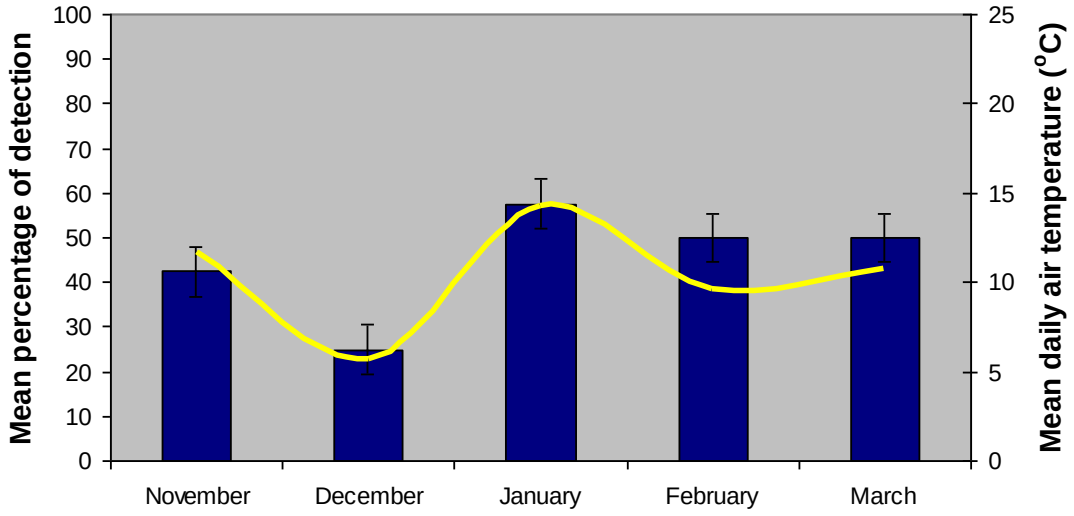


Figure 2.9. The effect of air temperature on detection of *Rhizoctonia solani* AG 2-2 LP in Zeon® zoysiagrass per 48-h sampling period each month from November 2012 to March 2013 at a driving range at The Club at Carlton Woods in The Woodlands, TX. The blue bars denote detection. The yellow trend line denotes air temperature.



Growth chamber study. *PCR analyses.* Strains used in this study were confirmed as *R. solani* and were further differentiated as the cultural type *R. solani* AG 2-2 LP, as universal primer pair ITS1-ITS4 and specific primer pair A091F-A091R amplified DNA fragments of approximately 740 bp and 350 bp, respectively (Figure 2.4).

Checks for natural infection and inoculation of zoysiagrass sod plugs. Zeon® zoysiagrass sod plugs collected from the field nursery at The Club at Carlton Woods were determined to be free of natural infection by *R. solani* AG 2-2 LP, as no hyphae were observed on toothpicks. Infestation of whole oat kernels was confirmed, as *R. solani* AG 2-2 LP hyphae were observed on kernels. By the first check after the start of incubation, *R. solani* AG 2-2 LP hyphae were observed on toothpicks that had been inserted into sod plugs and growing medium, confirming inoculation of all 12 pots with the infested whole oat kernels. By the second check after the start of incubation, fungal activity had progressed such that hyphae were observed on 43 of 48 toothpicks (89.6%) inserted into sod plugs and on 46 of 48 toothpicks (95.8%) inserted into growing medium (Table 2.3). The percentage of fungal activity was 100% in both sod plugs and growing medium in seven pots and 75% in both sod plugs and growing medium in one pot. The percentage of fungal activity in sod plugs and growing medium varied in the other four pots, with activity being greater in growing medium than in sod plugs in three of the pots. Conversely, all 12 pots that had not been inoculated were negative for *R. solani* AG 2-2 LP activity and continued to test negative throughout the study.

Table 2.3. *Rhizoctonia solani* AG 2-2 LP activity in Zeon® zoysiagrass sod plugs and in growing medium 10 d after inoculation with infested whole oat kernels. The mean percentage of detection was based on the number of four toothpicks inserted into plugs or soil that were colonized by the fungus.

Pot	Growing medium (# toothpicks)	Mean % detection (growing medium)	Sod plugs (# toothpicks)	Mean % detection (sod plugs)
1-1	3/4	75	2/4	50
1-2	4/4	100	4/4	100
1-3	4/4	100	4/4	100
1-4	4/4	100	3/4	75
2-1	3/4	75	3/4	75
2-2	4/4	100	4/4	100
2-3	4/4	100	4/4	100
2-4	4/4	100	4/4	100
3-1	4/4	100	3/4	75
3-2	2/4	50	4/4	100
3-3	4/4	100	4/4	100
3-4	4/4	100	4/4	100

Attachment of fungal hyphae to toothpicks in sod plugs versus growing medium. Despite a long photoperiod, sod plugs never established and began to decline in quality after incubation at 10°C during the first series of temperature treatments. Although *R. solani* AG 2-2 LP activity was detectable in both sod plugs and growing medium throughout both series of temperature treatments, it too diminished as the study

progressed (Tables 2.4 and 2.5). The effect of air temperature on attachment of *R. solani* AG 2-2 LP hyphae to toothpicks was significant in both sod plugs (F = 66.6, Df = 5, $P < 0.0001$) and growing medium (F = 10.9, Df = 5, $P < 0.0001$) in the first series of temperature treatments (Table 2.6) but in neither in the second series (sod plugs: F = 1.4, Df = 5, $P = 0.2378$; growing medium: F = 0.8, Df = 5, $P = 0.5482$) (Table 2.7). However, when data for the two series were combined, the effect was again significant in both sod plugs (F = 15.7, Df = 5, $P < 0.0001$) and growing medium (F = 6.9, Df = 5, $P < 0.0001$) (Table 2.8). A significant difference in treatments the first series occurred in both sod plugs and growing medium between the decrease in temperature from 20°C to 15°C (Table 2.4). Although attachment of hyphae to toothpicks in both sod plugs and growing medium was the greatest at 20°C, more hyphae attached to toothpicks in sod plugs than in growing medium. More hyphae also attached to toothpicks in sod plugs than in growing medium at 15°C. A significant difference in treatments also occurred in the combined series in both sod plugs and growing medium, but only between 20°C at the start of the series and all other decreasing and then increasing temperatures (Table 2.9). Although the effect of air temperature on attachment of hyphae to toothpicks was not significant in the second series of decreasing and then increasing temperature treatments, more attachment was observed at 20°C at the start of the series and in sod plugs (Table 2.5).

Table 2.4. Mean percentage of attachment and mean density of *Rhizoctonia solani* AG 2-2 LP hyphae on toothpicks inserted into growing medium and Zeon® zoysiagrass sod plugs in relation to a first series of decreasing (20°C, 15°C, 10°C) and then increasing (15°C, 20°C, 25°C) temperatures that simulate changing seasonal conditions in the field from fall (F) through spring (S). Means in the same column with the same letter are not significantly different at $P = 0.05$.

Treatment (°C)	% Attachment (growing medium)	Density (growing medium)	% Attachment (sod plugs)	Density (sod plugs)
20F	9.275a	0.9375a	18.442a	1.7292a
15F	2.892b	0.5000bc	4.450b	0.8125b
10F	0.175b	0.1250d	0.217c	0.2083d
15S	1.817b	0.6250b	1.500c	0.5208c
20S	1.983b	0.3542c	0.717c	0.2500cd
25S	0.225b	0.1042d	0.025c	0.0208d
$LSD_{0.05}$	2.8922	0.2257	2.4748	0.2745

Table 2.5. Mean percentage of attachment and mean density of *Rhizoctonia solani* AG 2-2 LP hyphae on toothpicks inserted into growing medium and Zeon® zoysiagrass sod plugs in relation to a second series of decreasing (20°C, 15°C, 10°C) and then increasing (15°C, 20°C, 25°C) temperatures that simulate changing seasonal conditions in the field from fall (F) through spring (S). Means in the same column with the same letter are not significantly different at $P = 0.05$.

Treatment (°C)	% Attachment (growing medium)	Density (growing medium)	% Attachment (sod plugs)	Density (sod plugs)
20F	0.2250a	0.16667a	0.3167a	0.16667a
15F	0.1083a	0.04167b	0.1667ab	0.12500a
10F	0.0333a	0.04167b	0.0333b	0.04167a
15S	0.2167a	0.10417ab	0.3083a	0.16667a

Table 2.5 Continued

Treatment (°C)	% Attachment (growing medium)	Density (growing medium)	% Attachment (sod plugs)	Density (sod plugs)
20S	0.1667a	0.08333ab	0.2167ab	0.14583a
25S	0.1500a	0.12500ab	0.0917ab	0.08333a
$LSD_{0.05}$	0.2253	0.1222	0.2737	0.1473

Table 2.6. The effect of air temperature on attachment and density of *Rhizoctonia solani* AG 2-2 LP hyphae on toothpicks inserted into growing medium and Zeon® zoysiagrass sod plugs in relation to a first series of decreasing (20°C, 15°C, 10°C) and then increasing (15°C, 20°C, 25°C) temperatures that simulate changing seasonal conditions in the field from fall through spring.

		Attachment (medium)		Density (medium)		Attachment (plugs)		Density (plugs)	
SOV	Df	MS	F value	MS	F value	MS	F value	MS	F value
Temperature	5	136.9	10.9***	1.2	15.8***	613.4	66.6***	4.6	41.0***

***P value < 0.0001

Table 2.7. The effect of air temperature on attachment and density of *Rhizoctonia solani* AG 2-2 LP hyphae on toothpicks inserted into growing medium and Zeon® zoysiagrass sod plugs in relation to a second series of decreasing (20°C, 15°C, 10°C) and then increasing (15°C, 20°C, 25°C) temperatures that simulate changing seasonal conditions in the field from fall through spring.

		Attachment (medium)		Density (medium)		Attachment (plugs)		Density (plugs)	
SOV	Df	MS	F value	MS	F value	MS	F value	MS	F value
Temperature	5	0.1	0.8¶	0.0	1.9‡	0.2	1.4†	0.0	0.9§

†P value = 0.2378; ‡P value = 0.2855; §P value = 0.4732; ¶P value = 0.5482

Table 2.8. The effect of air temperature on attachment and density of *Rhizoctonia solani* AG 2-2 LP hyphae on toothpicks inserted into growing medium and Zeon® zoysiagrass sod plugs in relation to a combined first and second series of decreasing (20°C, 15°C, 10°C) and then increasing (15°C, 20°C, 25°C) temperatures that simulate changing seasonal conditions in the field from fall through spring.

		Attachment (medium)		Density (medium)		Attachment (plugs)		Density (plugs)	
SOV	Df	MS	F value	MS	F value	MS	F value	MS	F value
Temperature	5	70.2	6.9***	0.7	7.4***	312.6	15.7***	2.6	12.4***

***P value < 0.0001

Table 2.9. Mean percentage of attachment and mean density of *Rhizoctonia solani* AG 2-2 LP hyphae on toothpicks inserted into growing medium and Zeon® zoysiagrass sod plugs in relation to a combined first and second series of decreasing (20°C, 15°C, 10°C) and then increasing (15°C, 20°C, 25°C) temperatures that simulate changing seasonal conditions in the field from fall (F) through spring (S). Means in the same column with the same letter are not significantly different at $P = 0.05$.

Treatment (°C)	% Attachment (growing medium)	Density (growing medium)	% Attachment (sod plugs)	Density (sod plugs)
20F	4.7500a	0.55208a	9.379a	0.9479a
15F	1.5000b	0.27083bc	2.308b	0.4688b
10F	0.1042b	0.08333d	0.125b	0.1250cd
15S	1.0167b	0.36458b	0.904b	0.3438bc
20S	1.0750b	0.21875bcd	0.467b	0.1979cd
25S	0.1875b	0.11458cd	0.058b	0.0521d
$LSD_{0.05}$	1.826	0.1785	2.5459	0.2596

Density of fungal hyphae attached to toothpicks in sod plugs versus growing medium. The effect of air temperature on density of *R. solani* AG 2-2 LP hyphae attached to toothpicks also was significant in both sod plugs ($F = 41.0$, $Df = 5$, $P < 0.0001$) and growing medium ($F = 15.8$, $Df = 5$, $P < 0.0001$) in the first series of temperature treatments (Table 2.6) but in neither in the second series (sod plugs: $F = 0.9$, $Df = 5$, $P = 0.4732$; growing medium: $F = 1.9$, $Df = 5$, $P = 0.2855$) (Table 2.7). However, when data for the two series were combined, the effect was again significant in both sod plugs ($F = 12.4$, $Df = 5$, $P < 0.0001$) and growing ($F = 7.4$, $Df = 5$, $P < 0.0001$) (Table 2.8). There were significant differences between treatments the first series in both sod plugs and growing medium except between the 10°C treatment in the downswing in temperature and the 25°C treatment in the upswing in temperature (Table 2.4). As with attachment of hyphae to toothpicks, density of hyphae was greatest in both sod plugs and growing medium at 20°C at the start of the series, and hyphae were denser in sod plugs than in growing medium. However, density in relation to increasing temperature treatments was greatest in both sod plugs and growing medium at 15°C, but hyphae were denser in growing medium than in sod plugs. In the combined series, there were significant differences between all temperature treatments in growing medium (Table 2.9). In sod plugs, there were significant differences between treatments except between the 10°C treatment in the downswing in temperature and the 20°C treatment in the upswing in temperature. As in the first series, density of hyphae in the combined series was greatest in both sod plugs and growing medium at 20°C at the start of the series, and hyphae were denser in sod plugs than in growing medium. Density in relation

to increasing temperature treatments in the combined series was greatest in both sod plugs and growing medium at 15°C, and hyphae were denser in growing medium than in sod plugs. Although the effect of air temperature on density of hyphae was not significant in the second series of temperature treatments, density in sod plugs was equally great at 20°C at the start of the series and at 15°C in the upswing in temperature (Table 2.5). Density of hyphae in growing medium was greatest at 20°C at the start of the series.

DISCUSSION

This study examined the effects of thatch/soil moisture and air temperature at a golf course driving range and the effect of air temperature in a growth chamber on *R. solani* AG 2-2 LP activity in Zeon® zoysiagrass. Previous studies have shown thatch/soil moisture to be an important environmental factor affecting development and severity of LP symptoms in zoysiagrass (Green et al. 1993, Obasa 2012, Obasa et al. 2013, Smiley et al. 2005). Therefore, we suspected that thatch/soil moisture also would be a factor affecting *R. solani* AG 2-2 LP activity prior to disease development. Thus, emphasis during the field study was placed on monitoring the volumetric water content in the top 7 cm of thatch/soil in the turfgrass. Previous studies also have shown thatch/soil temperature to be an important environmental factor affecting development and severity of LP symptoms in zoysiagrass (Green et al. 1993, Obasa 2012, Obasa et al. 2013, Smiley et al. 2005). Therefore, we also suspected that thatch/soil temperature would be a factor affecting *R. solani* AG 2-2 LP activity prior to disease development. But rather than focus on this temperature, we hypothesized that air temperature influences

thatch/soil temperature, and thus it may be used along with thatch/soil moisture to predict fungal activity prior to disease development.

The effects of thatch/soil moisture and air temperature on seasonal *R. solani* AG 2-2 LP activity in zoysiagrass at the driving range were inferred through simple linear regression analyses. As expected, both thatch/soil moisture and air temperature were significant environmental factors affecting fungal activity, particularly when they averaged between 28.3% and 53.3%, and between 9.2°C and 20.8°C, respectively. As the driving range and other previous studies showed thatch/soil moisture to be an important environmental factor in development and severity of LP symptoms in zoysiagrass, moisture was monitored in pots during both series of temperature treatments in the growth chamber study. The mean percentage of moisture in pots during the study was approximately 56%. The effect of air temperature on *R. solani* AG 2-2 LP activity was consistent with the known range of thatch/soil temperatures (10°C to 21°C) when LP symptoms occur (Tredway and Burpee 2001). Thus, air temperature may be used to infer thatch/soil temperature and subsequently predict fungal activity prior to disease development. However, results of the driving range study are contrary to laboratory study results by Green et al. (1993) that indicate thatch/soil temperatures between 15°C and 25°C favor the development of LP in zoysiagrass. Green et al. (1993) also showed that development of LP is suppressed when thatch/soil temperature exceeds 30°C. This temperature is indicative of the summer months when *R. solani* AG 2-2 LP is not active, except when cool and wet weather occurs. The findings by Green et al. (1993) are contrary to those by Obasa et al. (2013), who noted the absence of LP symptoms in

summer field studies in Kansas when recorded thatch/soil temperatures were between 28°C and 29°C. Obasa et al. (2013) concluded that the absence of LP symptoms in the summer was not directly due to thatch/soil temperatures that hinder growth of the fungus while promoting growth of turfgrass roots and shoots, as Green et al. (1993) had suggested, but rather alteration in gene expression in actively growing zoysiagrass at high temperatures may influence susceptibility to LP.

Although data analyses in the driving range study indicate that both thatch/soil moisture and air temperature are significant environmental factors affecting *R. solani* AG 2-2 LP activity in zoysiagrass, it became apparent that air temperature may be more important, especially when the driving range is routinely irrigated. LP symptom development during the fall in 2011 occurred in mid-November, which was about five weeks later than in 2012 and about three weeks later than in 2013. Delayed LP symptom development during the fall in 2011 was more likely influenced by the fact that the preceding summer was the warmest recorded in Texas by the National Oceanic and Atmospheric Administration's National Climatic Data Center.

There is a lack of research that would elucidate and differentiate the mechanisms responsible for observed differences in development and severity of LP symptoms within zoysiagrass genotypes, and especially those mechanisms influenced by environmental factors. Various university cooperative extension, golf and athletic field association, and turfgrass industry reports summarize research initiatives that are overseen by the U.S. Department of Agriculture's National Turfgrass Evaluation Program and that evaluate zoysiagrass genotypes developed to improve such

economically important traits as overall quality, leaf color and texture, frost tolerance, spring green-up, recovery from divot injury, and seed head formation. Environmental conditions in the field may not be uniform or constant long enough to evaluate in terms of the effects on *R. solani* AG 2-2 LP activity and on development and severity of LP symptoms in zoysiagrass genotypes. Air temperature fluctuates during any given day and from day to day, week to week, month to month, and year to year. Thus, controlled temperature studies are needed to evaluate the seasonal dynamics of *R. solani* AG 2-2 LP activity in zoysiagrass genotypes.

Interestingly, during the growth chamber study, *R. solani* AG 2-2 LP tended to be more active overall in Zeon® zoysiagrass sod plugs when pots were subjected to fall-like temperatures, and more active overall in growing medium when pots were subjected to spring-like temperatures. However, in relation to only sod plugs, more fungal activity was observed at 20°C when pots were subjected to fall-like temperatures and at 15°C when pots were subjected to spring-like temperatures. In relation to only growing medium, more fungal activity was observed at 20°C when pots were subjected to both fall-like and spring-like temperatures. Data on attachment and density of *R. solani* AG 2-2 LP hyphae on toothpicks in both series of temperature treatments seem to indicate that fungal activity shifts from sod plugs to growing medium and then back to sod plugs in relation to seasonal transitions in temperature, particularly between winter (10°C) and spring (15°C), and between summer (25°C) and fall (20°C). If so, this may be due to a survival mechanism in which the fungus seeks more favorable environmental conditions during the colder and hotter months. It is known that the fungus colonizes soil in a linear

fashion (Kumar et al. 1999). It is feasible that the fungus also colonizes soil vertically in relation to temperature. This was observed somewhat in both the driving range and growth chamber studies in terms of where on toothpicks hyphae attached. Hyphae tended to attach higher on the portion of toothpicks located more in thatch when temperatures were favorable for fungal activity, and to attach lower on the portion of toothpicks located more in soil when temperatures were above (hotter) or below (colder) those favorable for fungal activity.

The shift in fungal activity between sod plugs and growing medium and vice versa could also indicate the importance of the host in the interaction. It is feasible that changes in gene expression in zoysiagrass entering or exiting dormancy may be sensed by the fungus and trigger movement as well as infection of the host. Both the quality of the sod plugs and activity of *R. solani* AG 2-2 LP in sod plugs and growing medium declined as the study progressed. It is possible that the amount of inoculum resulted in diseased sod that failed to recover. The 10g of infested oat kernels used for inoculation far exceeded the 10 kernels in a method developed by Tisserat et al. (1989) and used in a growth chamber study by Obasa et al. (2012) to evaluate the susceptibility to LP of 14 new freeze-tolerant zoysiagrass progeny. It also is possible that the 16-h photoperiod failed to provide adequate light to compensate for the decrease in temperature that would hinder growth of the sod plugs (Gelernter and Stowell 2005, Glenn 2013, Woods 2013). Temperature and light are among the most important environmental factors affecting plant growth (Woods 2013). A function of light intensity (moles of photons per square meter per second) and duration (time in seconds), the daily light integral (DLI) is a

measurement used by growers to quantify the light needed during the course of a day to produce a crop in a greenhouse. Preliminary research conducted by the University of Florida indicates zoysiagrass has a lower DLI requirement than other species of warm-season turfgrasses, with values ranging from 10.5 to 12.0 $mol/m^{-2}/d^{-1}$ (Glenn 2013). However, based on an average light intensity of 86.6 $mol/m^{-2}/s^{-1}$, measured using a LI-189 light meter (LI-COR, Inc., Lincoln, NE), and the 16-h photoperiod, the DLI in the growth chamber was 5.0 $mol/m^{-2}/d^{-1}$. This would explain the observed decline in sod plugs but it does not explain the decline in fungal activity in both sod plugs and growing medium. But it does suggest *R. solani* AG 2-2 LP activity is influenced by the growth of the turfgrass, which in turn is influenced by temperature and light. Perhaps day length in addition to temperature is a trigger for changes in gene expression in zoysiagrass entering or exiting dormancy and thus a trigger for increased or decreased fungal activity as well.

CHAPTER III

EFFECTS OF PRIOR SUMMERTIME IRRIGATION AND NITROGEN ON THATCH/SOIL MOISTURE AND TEMPERATURE, AND ON SEVERITY OF LARGE PATCH IN ZOYSIAGRASS

INTRODUCTION

A preference for zoysiagrass (*Zoysia* Willd.) on golf course fairways and in the landscape is increasing across the southern United States, and especially in Texas, due to its aesthetic appeal (medium to fine texture and rich green color), excellent heat and drought tolerance, good shade and cold tolerance, and low requirement for cultural inputs, such as fertilizer and pesticide (Beard 1972, Chandra et al. 2014, Duble 1989, Fry and Huang 2004, Fry et al. 2008, NTEP 2007, Okeyo et al. 2011, Patton and Reicher 2007, Wherley et al. 2011). But despite these desirable characteristics, a primary limitation of this warm-season turfgrass is its susceptibility to large patch (LP), caused by the soil-borne fungus *Rhizoctonia solani* AG 2-2 LP (Green et al. 1993, Hyakumachi et al. 1998, Smiley et al. 2005).

LP is a serious disease that weakens turfgrass and reduces its quality, and kills it in a severe infection (Toda et al. 2004). Actively growing turfgrass tends not to exhibit disease symptoms, and LP often occurs when turfgrass is entering or exiting winter dormancy in the fall and the spring, respectively, and when the weather is generally cool and wet (Emmons 2000, Green et al. 1993, Martinez et al. 2009). Symptoms of LP in the fall include circular patches of blighted turfgrass, ranging in diameter from less than one

meter to up to nearly eight meters (Green et al. 1993, Martinez et al. 2009, Tisserat et al. 1994) (Figure 2.1). Leaf blades at the patch perimeter initially appear yellow, orange or reddish brown in color but then become tan and collapse to the ground, forming a mat (Green et al. 1993, Merrill 2011). Symptoms in the spring include thin, sunken light brown patches in turfgrass that are slower to break dormancy and may be invaded by weeds (Figure 2.1).

Cultural mismanagement of zoysiagrass in the summer can result in a decline in quality that may make the turfgrass more susceptible to LP in the following fall and spring. There is no zoysiagrass cultivar known to be resistant to LP, and once established in the soil the causal agent cannot be eliminated. Thus, the only recourse to delay and/or suppress incidence and severity of the disease is through cultural and chemical management prior to the onset of symptoms. Determining the best management practices for zoysiagrass is necessary to achieve acceptable quality in the summer, with the least effect on development and severity of LP in the following fall and spring. Management of LP is of particular concern to homeowners, and turfgrass producers and managers, who encounter economic loss due to blighting or dead turfgrass.

Environmental conditions, such as moisture and temperature, have been shown to be important factors in the development and severity of LP symptoms in zoysiagrass (Green et al. 1993, Obasa 2012, Obasa et al. 2013, Smiley et al. 2005). However, knowledge is still lacking regarding the influence of summertime cultural inputs on quality of zoysiagrass and on development and severity of LP in the following fall and spring in Texas. Thus, the objective of this study was to determine the effects of

summertime irrigation and nitrogen levels on turfgrass quality, thatch/soil moisture, thatch/soil temperature, *R. solani* AG 2-2 LP activity and LP symptom development.

MATERIALS AND METHODS

Design and treatments of experimental plots. This study was conducted from May 2012 to April 2014 in an established stand (60.9 m x 15.2 m) of 'Palisades' zoysiagrass (*Z. japonica* Steud.) (Texas A&M AgriLife Research, Dallas, TX) at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Soil type at the site is a Boonville fine sandy loam. Sixty-four experimental plots were set up as a split-plot, randomized complete block design with four replications per 16 combinations of treatments (Figure 3.1). Main plot (6.1 m x 6.1 m) factor was irrigation level, which consisted of a percentage of historical reference evapotranspiration (0, 36, 60 or 100% of ETo) (Texas ET Network, http://texaset.tamu.edu/). Sub plot (6.1 m x 1.5 m) factor was nitrogen level (0, 49, 98 or 196 kg ha^{-1}). Irrigation was applied three times a week (Tuesday, Thursday and Saturday) during each growing season. Nitrogen in the form of quick-release ammonium sulfate (21-0-0) was divided into three applications and applied using a drop spreader at six-week intervals beginning in May. Also during each growing season, height of the turfgrass was maintained at 1.27 cm by mowing three times a week (Monday, Wednesday and Friday) with a triplex reel mower, with clippings returned.

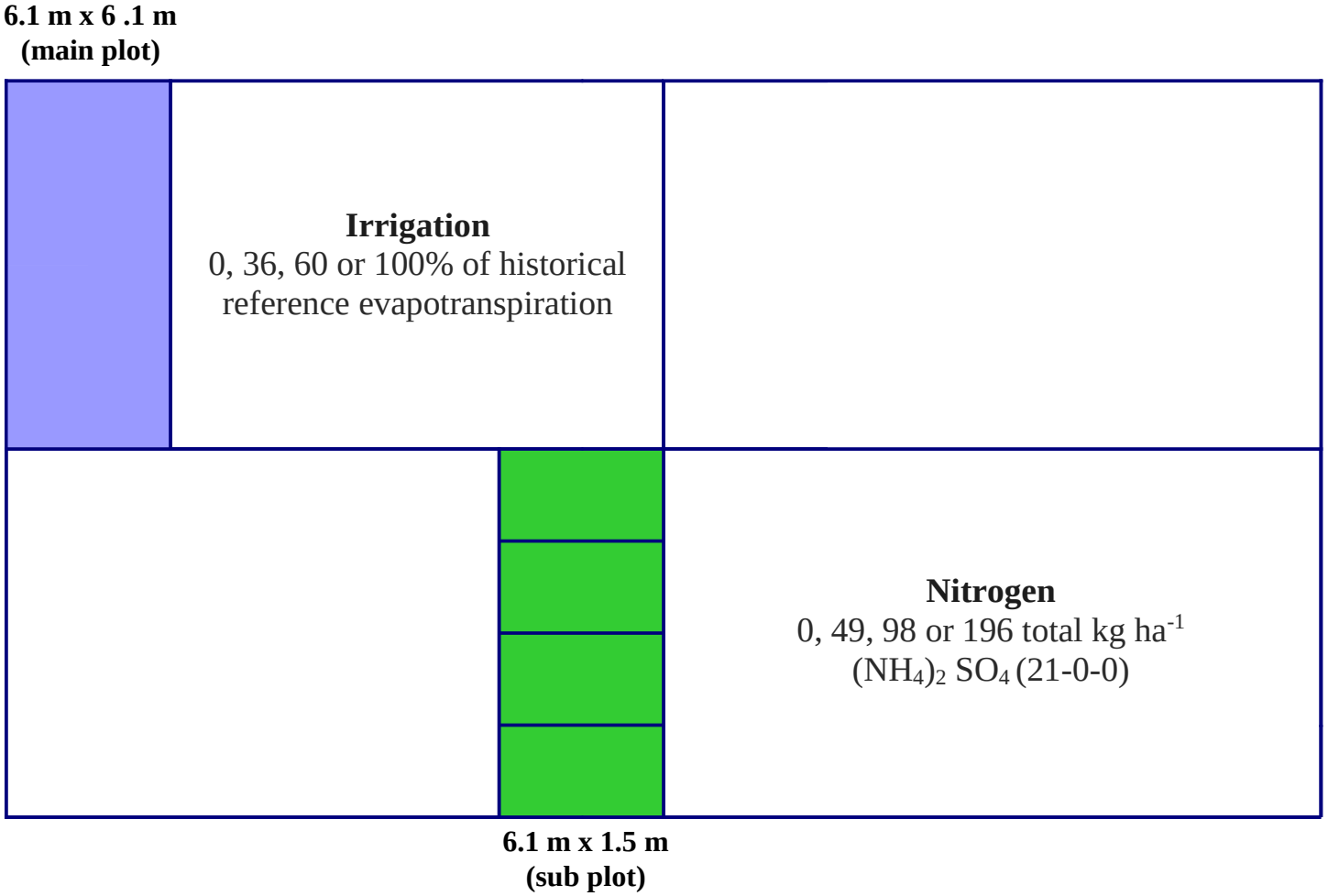
Monitoring of environmental conditions. Volumetric water content in the top 7 cm of thatch/soil in the center of each experimental plot and the mean daily air temperature for the location were measured as previously described in a driving range

study in Chapter II. A Traceable® Lollipop™ Shockproof/Water-resistant Thermometer (Control Company, Friendswood, TX) that was inserted into the turfgrass to a depth of 7 cm was used to measure the thatch/soil temperature in the center of each experimental plot. Measurements were taken once each month for approximately 1 minute at the beginning of a 48-h sampling period for *R. solani* AG 2-2 LP.

Assessment of turfgrass quality. Zoysiagrass in each experimental plot was visually rated for turfgrass quality, using a scale of 1 to 9 developed by the National Turfgrass Evaluation Program (http://www.ntep.org/pdf/ratings.pdf) that was based on color, density, uniformity, texture, disease and environmental stress, whereby 1 = poorest or brown and dead; 5 = minimally acceptable; and 9 = perfect or ideal. Ratings were conducted on June 1, June 22, July 6, July 24, August 5 and October 10 in 2012, and on June 11, June 28, August 8, August 22 and October 2 in 2013.

Assessment of *R. solani* AG 2-2 LP activity and LP symptom development. The 64 experimental plots contained naturally occurring populations of *Rhizoctonia* spp. and were not treated for LP with any fungicides. A toothpick baiting method previously described in the driving range study in Chapter II was used to sample the center of each experimental plot monthly for *R. solani* AG 2-2 LP. Plots were scored for *R. solani* AG 2-2 LP activity based on the absence (0) or presence (1) on toothpicks of LP hyphae. Plots were visually assessed each month for LP development based on the percentage of area that exhibited symptoms.

Figure 3.1. Design of 64 experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory. A 60.9 m x 15.2 m area was divided into four blocks, with each block being further divided into four (6.1 m x 6.1 m) main plots (depicted in blue) and each main plot being further divided into four (6.1 m x 1.5 m) sub plots (depicted in green). Each block contained 16 combinations of treatments. Main plot factor was irrigation level. Sub plot factor was nitrogen level. Irrigation was applied three times a week during each growing season. Nitrogen was divided into three applications and applied at six-week intervals during each growing season.



Statistical analyses. To determine the effects of summertime irrigation and nitrogen levels on turfgrass quality, scores for each rating period were subjected to Analysis of Variance (ANOVA) using the PROC GLM procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC). Data were analyzed separately with respect to year (2012-2013 or 2013-2014). SAS code used in analyses is shown in Appendix E. Means of significant independent variables were separated using Fisher's protected least significant difference (LSD) test at $P = 0.05$. To determine the effects of summertime irrigation and nitrogen levels on thatch/soil moisture and temperature, *R. solani* AG 2-2 LP activity and LP symptom development, data for the sampling periods from November to March, when the fungus was speculated to be active, were subjected to ANOVA using the PROC GLM procedure in SAS version 9.3. The five sampling periods in both years were analyzed individually. SAS code used in analyses is shown in Appendix F. Means of significant independent variables were separated using the LSD test at $P = 0.05$. To determine the effects of thatch/soil temperature and air temperature (independent variables) on *R. solani* AG 2-2 LP activity (dependent variable), data for the sampling periods from November to March were subjected to regression analysis between dependent and independent variables using the REG procedure in SAS version 9.4. Data were analyzed with respect to year (2012-2013 or 2013-2014) as well as the study as a whole (2012-2014). SAS code used in analyses is shown in Appendix G.

RESULTS

The effects of summertime irrigation and nitrogen levels on turfgrass quality. The effect of summertime irrigation level on the quality of zoysiagrass was

significant during all rating dates in both years, whereas the effect of summertime nitrogen level was significant during three of the six rating dates in the first year and none of the five rating dates in the second year (Table 3.1). Acceptable turfgrass was achieved with an irrigation level of 100% of ETo during all rating dates in both years (Table 3.2). An irrigation level of 60% of ETo also produced acceptable turfgrass during all rating dates in the first year and during four of the five rating dates in the second year. An irrigation level of 36% of ETo produced acceptable turfgrass during five of the six rating dates in the first year and during one of the five rating dates in the second year. The quality of turfgrass was not significantly different when irrigated at 100%, 60% or 36% of ETo in the first year or at 100% or 60% of ETo in the second year. Acceptable turfgrass was achieved with all nitrogen levels during four of the six rating dates in the first year and during one of the five rating dates in the second year (Table 3.3). Turfgrass quality was significantly improved with the application of nitrogen, but only early in the growing season. However, there were no significant differences in turfgrass quality between the 196 kg ha^{-1} and 98 kg ha^{-1} nitrogen levels.

Table 3.1. The effects of irrigation and nitrogen levels on quality of 64 experimental plots of 'Palisades' zoysiagrass at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Plots were visually rated six times in 2012 and five times in 2013 using a scale developed by the National Turfgrass Evaluation Program. NS = turfgrass quality was rated, but no statistical output was generated during analyses.

2012

	Irrigation			Nitrogen		
Rating date	Df	F value	*P* value	Df	F value	*P* value
June 1	3	112.8	< 0.0001	3	5.6	0.0030
June 22	3	235.9	< 0.0001	3	2.7	0.0583
July 6	3	248.7	< 0.0001	3	11	< 0.0001
July 24	3	246.2	< 0.0001	3	1.6	0.2175
August 5	3	623.7	< 0.0001	3	1.2	0.3127
October 10	3	31.7	< 0.0001	3	1.3	0.2787

2013

	Irrigation			Nitrogen		
Rating date	Df	F value	*P* value	Df	F value	*P* value
June 11	3	256.9	< 0.0001	3	2.5	0.0760
June 28	3	485.5	< 0.0001	3	1.9	0.1475
August 8	3	759.1	< 0.0001	3	1.8	0.1646
August 22	3	∞	< 0.0001	3	NS	NS
October 2	3	550.1	< 0.0001	3	0.9	0.4176

Table 3.2. Mean quality of turfgrass in relation to irrigation level per rating date at 64 experimental plots of 'Palisades' zoysiagrass at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Plots were visually rated six times in 2012 and five times in 2013 using a scale from 1 to 9 developed by the National Turfgrass Evaluation Program, whereby 1 = poorest or dead; 5 = minimally acceptable; and 9 = perfect. Means in the same column with the same letter are not significantly different at $P = 0.05$.

2012

		Treatment			
Rating date	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
June 1	0.4731	2.2188c	4.5938b	5.7500a	6.0938a
June 22	0.271	3.5313c	6.6250a	6.3750ab	6.2500b
July 6	0.3126	2.6875b	6.1563a	6.0938a	6.1250a
July 24	0.2376	3.2188b	5.6875a	5.8750a	5.8750a
August 5	0.2639	1.7500c	6.1875b	6.2500b	6.5625a
October 10	0.4909	4.7500b	6.8125a	6.5625a	6.6250a

2013

		Treatment			
Rating date	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
June 11	0.3112	2.0625d	4.9375c	5.4063b	5.9688a
June 28	0.2513	1.7500c	4.5313b	5.7813a	5.9063a
August 8	0.2004	1.50000d	3.50000c	4.62500b	6.06250a
August 22	0	1.000d	4.000c	5.250b	6.000a
October 2	0.3446	1.1875c	6.3750b	7.0000a	7.0000a

Table 3.3. Mean quality of turfgrass in relation to nitrogen level per rating date at 64 experimental plots of 'Palisades' zoysiagrass at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Plots were visually rated six times in 2012 and five times in 2013 using a scale from 1 to 9 developed by the National Turfgrass Evaluation Program, whereby 1 = poorest or dead; 5 = minimally acceptable; and 9 = perfect. Means in the same column with the same letter are not significantly different at $P = 0.05$.

2012

		Treatment			
Rating date	$LSD_{0.05}$	0 kg ha^{-1} N	49 kg ha^{-1} N	98 kg ha^{-1} N	196 kg ha^{-1} N
June 1	0.4731	4.1563b	4.5625ab	5.0000a	4.9375a
June 22	0.271	5.5938ab	5.5313b	5.8438a	5.8125a
July 6	0.3126	4.8438b	5.0938b	5.5000a	5.6250a
July 24	0.2376	5.0938a	5.2188a	5.2813a	5.0625a
August 5	0.2639	5.0625a	5.3125a	5.1875a	5.1875a
October 10	0.4909	6.0000a	6.60625a	6.4375a	6.2500a

2013

		Treatment			
Rating date	$LSD_{0.05}$	0 kg ha^{-1} N	49 kg ha^{-1} N	98 kg ha^{-1} N	196 kg ha^{-1} N
June 11	0.3112	4.3750b	4.5625ab	4.6563ab	4.7813a
June 28	0.2513	4.3125a	4.5625ab	4.5313a	4.5625a
August 8	0.2004	4.00000a	4.00000a	3.87500a	3.81250a
August 22	0	4.063a	4.063a	4.063a	4.063a
October 2	0.3446	5.3125a	5.3125a	5.5625a	5.3750a

Table 3.4. The effects of irrigation and nitrogen levels on mean percentage of thatch/soil moisture per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX.

2012-2013						
	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	150	< 0.0001	3	0.4	0.7428
December	3	48.6	< 0.0001	3	1	0.4005
January	3	149.1	< 0.0001	3	1.6	0.1935
February	3	248.7	< 0.0001	3	0.8	0.4816
March	3	165.6	< 0.0001	3	1.9	0.1354
2013-2014						
	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	58.9	< 0.0001	3	0.4	0.7504
December	3	147.9	< 0.0001	3	0.5	0.6530
January	3	84.3	< 0.0001	3	0.8	0.5042
February	3	117.4	< 0.0001	3	0.7	0.5304
March	3	26.1	< 0.0001	3	2.1	0.1038

The effects of summertime irrigation and nitrogen levels on thatch/soil moisture. The effect of summertime irrigation level on thatch/soil moisture in the later

season was significant (Table 3.4). There were significant differences between thatch/soil moisture caused by summertime irrigation levels in both years. The greatest mean percentage of thatch/soil moisture was achieved with 60% and 100% of ETo (Table 3.5). Summertime nitrogen level did not affect thatch/soil moisture in the later season (Table 3.4).

The effects of summertime irrigation and nitrogen levels on thatch/soil temperature. The effect of summertime irrigation level on thatch/soil temperature in the later season was significant during the five sampling periods from November to March in the first year and during four (November, January, February and March) of the sampling periods in the second year (Table 3.6). In both years, an irrigation level of 0% of ETo resulted in the highest mean thatch/soil temperature (Table 3.7). The effect of summertime nitrogen level on thatch/soil temperature in the later season was not significant except for a few sampling periods (Table 3.6). There were significant differences between thatch/soil temperatures caused by nitrogen levels, but there was no consistent trend (Table 3.8).

Table 3.5. Mean percentage of thatch/soil moisture in relation to irrigation level per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$.

2012-2013

		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	1.3218	20.4813d	26.5063c	31.4313b	33.1250a
December	1.3532	16.7938c	20.4656b	23.4438a	24.1875a
January	1.1217	27.4406c	34.3500b	37.3750a	38.1688a
February	1.1671	24.3500d	34.0188c	37.5406b	38.8781a
March	1.5035	19.1063c	29.2031b	32.9344a	34.4125a

2013-2014

		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	1.4866	20.2719c	21.5281c	26.0813b	29.0500a
December	1.3241	21.1469d	25.7750c	29.9563b	34.5688a
January	1.2819	18.9500d	22.7000c	26.3563b	28.4875a
February	1.2825	29.8375d	34.0750c	38.4219b	41.1125a
March	1.6296	16.7125c	16.6469c	19.7625b	22.8813a

Table 3.6. The effects of irrigation and nitrogen levels on thatch/soil temperature per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX.

2012-2013						
	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	30.1	< 0.0001	3	1.2	0.3053
December	3	5.9	0.0009	3	0.5	0.6946
January	3	59.7	< 0.0001	3	0.7	0.5384
February	3	4.4	0.0059	3	0.8	0.5191
March	3	54.1	< 0.0001	3	2.7	0.0522
2013-2014						
	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	47.3	< 0.0001	3	1.1	0.3629
December	3	2.2	0.0898	3	2.4	0.0692
January	3	61.2	< 0.0001	3	2.7	0.0479
February	3	86.2	< 0.0001	3	3.2	0.0264
March	3	74.3	< 0.0001	3	1.7	0.1693

Table 3.7. Mean thatch/soil temperature (°C) in relation to irrigation level per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$. NS = large patch symptoms were recorded, but no statistical output was generated during analyses.

2012-2013

		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	0.4318	24.4313a	22.6375b	22.8938b	22.8188b
December	0.1583	19.10000a	18.87813b	18.77500b	18.87500b
January	0.186	12.57500a	11.45625c	11.54375bc	11.72813b
February	0.1335	11.30625a	11.25000ab	11.14062bc	11.08750c
March	0.2518	22.2250a	20.9500bc	20.7406c	21.1594b

2013-2014

		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	NS	NS	NS	NS	NS
December	0.1998	16.6906b	16.7531ab	16.9094a	16.6781b
January	0.322	13.1344a	11.9188b	11.0188d	11.5563c
February	0.085	7.33750d	7.56563c	7.97813a	7.81563b
March	0.3464	21.3938a	19.8406b	18.9031d	19.4906c

Table 3.8. Mean thatch/soil temperature (°C) in relation to nitrogen level per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$. NS = large patch symptoms were recorded, but no statistical output was generated during analyses.

2012-2013

		Treatment			
Sampling date	$LSD_{0.05}$	0 kg ha^{-1}	49 kg ha^{-1}	98 kg ha^{-1}	196 kg ha^{-1}
November	0.4318	23.0313a	23.4188a	23.2250a	23.1063a
December	0.1583	18.91250a	18.86875a	18.88750a	18.95938a
January	0.186	11.88438a	11.85625a	11.80625a	11.75625a
February	0.1335	11.19063a	11.16563a	11.17188a	11.25625a
March	0.2518	21.4563a	21.2438ab	21.1000b	21.2750ab

2013-2014

		Treatment			
Sampling date	$LSD_{0.05}$	0 kg ha^{-1}	49 kg ha^{-1}	98 kg ha^{-1}	196 kg ha^{-1}
November	NS	NS	NS	NS	NS
December	0.1998	16.6188b	16.8344a	16.8594a	16.7188a
January	0.322	12.1281a	11.9875ab	11.6969b	11.8156ab
February	0.085	7.60000b	7.66875ab	7.72188a	7.70625a
March	0.3464	20.0625a	20.0219a	19.7250a	19.8188a

The effects of summertime irrigation and nitrogen levels on *R. solani* AG 2-2 LP activity. The effect of summertime irrigation level on *R. solani* AG 2-2 LP activity in the later season was not significant during any of the five sampling periods in the first year but was significant during November and January in the second year (Table 3.9). There were significant differences between irrigation levels during the two sampling periods, with 100% of ETo resulting in the greatest fungal activity (Table 3.10). The effect of summertime nitrogen level on *R. solani* AG 2-2 LP activity in the later season was not significant during any sampling period in either year (Table 3.9).

Table 3.9. The effects of irrigation and nitrogen levels on *Rhizoctonia solani* AG 2-2 LP activity per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX.

2012-2013						
	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	1.2	0.3248	3	1	0.4059
December	3	0.4	0.7578	3	1.5	0.2111
January	3	0.9	0.4324	3	0.9	0.4324
February	3	0.5	0.6544	3	0.4	0.7222
March	3	1.1	0.3681	3	0.1	0.9429

Table 3.9 Continued

2013-2014						
	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	2.6	0.054	3	0.4	0.7786
December	3	0.2	0.8696	3	1	0.3819
January	3	4.1	0.0087	3	0.5	0.6544
February	3	1.9	0.1307	3	1.4	0.2615
March	3	1.3	0.287	3	0.2	0.8824

Table 3.10. Mean detection score of *Rhizoctonia solani* AG 2-2 LP in relation to irrigation level per 48-h sampling period from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$.

2012-2013					
		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	0.3267	0.5000a	0.5000a	0.5000a	0.2500a
December	0.242	0.5625a	0.5625a	0.5938a	0.4688a
January	0.2414	0.3438a	0.3125a	0.5000a	0.4063a
February	0.228	0.5000a	0.3750a	0.3750a	0.4375a
March	0.2341	0.6875a	0.5625a	0.6563a	0.5000a

Table 3.10 Continued

2013-2014					
		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	0.2178	0.4375b	0.3750b	0.5625ab	0.6563a
December	0.1797	0.15625a	0.15625a	0.15625a	0.21875a
January	0.114	0.03125b	0.00000b	0.06250b	0.18750a
February	0.0948	0.00000a	0.06250a	0.00000a	0.09375a
March	0.2414	0.4375a	0.3750a	0.5938a	0.5313a

The effects of summertime irrigation and nitrogen levels on LP symptom development. The effect of summertime irrigation level on LP symptom development in the later season was significant during two (November and January) of the five sampling periods in the first year and during three (November, December and March) of the sampling periods in the second year (Table 3.11). An irrigation level of 60% of ETo in the first year and of 60% of ETo and 100% of ETo in the second year resulted in the greatest mean percentage of LP symptom development (Table 3.12). The effect of summertime nitrogen level on LP symptom development in the later season was not significant except for one (March) of the sampling periods in the second year when the applications reduced disease symptoms compared to the non-treated control (Tables 3.11 and 3.13).

Table 3.11. The effects of irrigation and nitrogen levels on large patch symptom development per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. NR = no large patch symptoms were recorded, and thus no statistical data was generated.

2012-2013

	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	24.9	< 0.0001	3	1.3	0.2783
December	3	NR	NR	3	NR	NR
January	3	65	< 0.0001	3	2.5	0.0625
February	3	NR	NR	3	NR	NR
March	3	29.9	< 0.0001	3	1.1	0.3597

2013-2014

	Irrigation			Nitrogen		
Sampling month	Df	F value	*P* value	Df	F value	*P* value
November	3	9.9	< 0.0001	3	0.4	0.7817
December	3	44.2	< 0.0001	3	0.9	0.4585
January	3	NR	NR	3	NR	NR
February	3	NR	NR	3	NR	NR
March	3	35.4	< 0.0001	3	2.7	0.0493

Table 3.12. Mean percentage of large patch symptom development in relation to irrigation level per 48-h sampling period from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$. NR = no large patch symptoms were recorded, and thus no statistical data was generated. NS = large patch symptoms were recorded, but no statistical output was generated during analyses.

2012-2013

		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	8.9793	3.750c	12.500bc	40.625a	19.375b
December	NR	NR	NR	NR	NR
January	9.6309	1.250d	20.156c	64.063a	45.625b
February	NR	NR	NR	NR	NR
March	NS	NS	NS	NS	NS

2013-2014

		Treatment			
Sampling month	$LSD_{0.05}$	0% of ETo	36% of ETo	60% of ETo	100% of ETo
November	5.4938	12.656b	5.813c	20.531a	10.094bc
December	8.2984	0.156d	8.594c	29.375b	43.438a
January	NR	NR	NR	NR	NR
February	NR	NR	NR	NR	NR
March	9.9448	1.719c	41.094ab	31.563b	50.313a

Table 3.13. Mean percentage of large patch symptom development in relation to nitrogen level per 48-h sampling period from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$. NR = no large patch symptoms were recorded, and thus no statistical data was generated. NS = large patch symptoms were recorded, but no statistical output was generated during analyses.

2012-2013

		Treatment			
Sampling date	$LSD_{0.05}$	0 kg ha^{-1}	49 kg ha^{-1}	98 kg ha^{-1}	196 kg ha^{-1}
November	8.9793	18.125a	22.500a	21.250a	14.375a
December	NR	NR	NR	NR	NR
January	9.6309	27.500b	35.000ab	39.375a	29.219b
February	NR	NR	NR	NR	NR
March	NS	NS	NS	NS	NS

2013-2014

		Treatment			
Sampling date	$LSD_{0.05}$	0 kg ha^{-1}	49 kg ha^{-1}	98 kg ha^{-1}	196 kg ha^{-1}
November	5.4938	13.719a	12.188a	10.844a	12.344a
December	8.2984	24.063a	18.594a	17.969a	20.938a
January	NR	NR	NR	NR	NR
February	NR	NR	NR	NR	NR
March	9.9448	39.844a	27.344b	29.219b	28.281b

***R. solani* AG 2-2 LP activity in relation to air temperature and thatch/soil temperature.** The effect of air temperature on *R. solani* AG 2-2 LP activity was not significant during the five sampling periods that the fungus was active (November to March) in the first year but was significant during those sampling periods in the second year. The effect of thatch/soil temperature on *R. solani* AG 2-2 LP activity was significant during the five sampling periods that the fungus was active (November to March) in both years ($P = 0.0155$ and $P = 0.0002$, respectively). Fungal activity tended to increase during the fall, recede during the winter, and again increase in the spring with a seasonal change in air and thatch/soil temperatures. Fungal activity again decreased and eventually became undetectable by late spring once temperatures increased beyond 20°C. *R. solani* AG 2-2 LP activity in the first year was observed when the mean daily air temperature was between 11.1°C and 22.0°C, and the mean thatch/soil temperature was between 11.2°C and 23.2°C (Figure 3.2) (Table 3.14). Fungal activity in the second year was observed when the mean daily air temperature was between 3.3°C and 16.6°C, and the mean thatch/soil temperature was between 7.7°C and 19.9°C (Figure 3.2) (Table 3.14). The greatest activity in the fall was observed when the thatch/soil temperature averaged between 12.7°C and 18.9°C, and when the air temperature averaged between 15.9°C and 19.6°C. The greatest activity in the spring was observed when the thatch/soil temperature averaged between 19.9°C and 21.3°C, and when the air temperature averaged between 16.0°C and 16.6°C.

Table 3.14. Mean percentage of detection of *Rhizoctonia solani* AG 2-2 LP in relation to mean daily air temperature and mean thatch/soil temperature per 48-h sampling period from November 2012 to March 2013 and from November 2013 to March 2014 at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX.

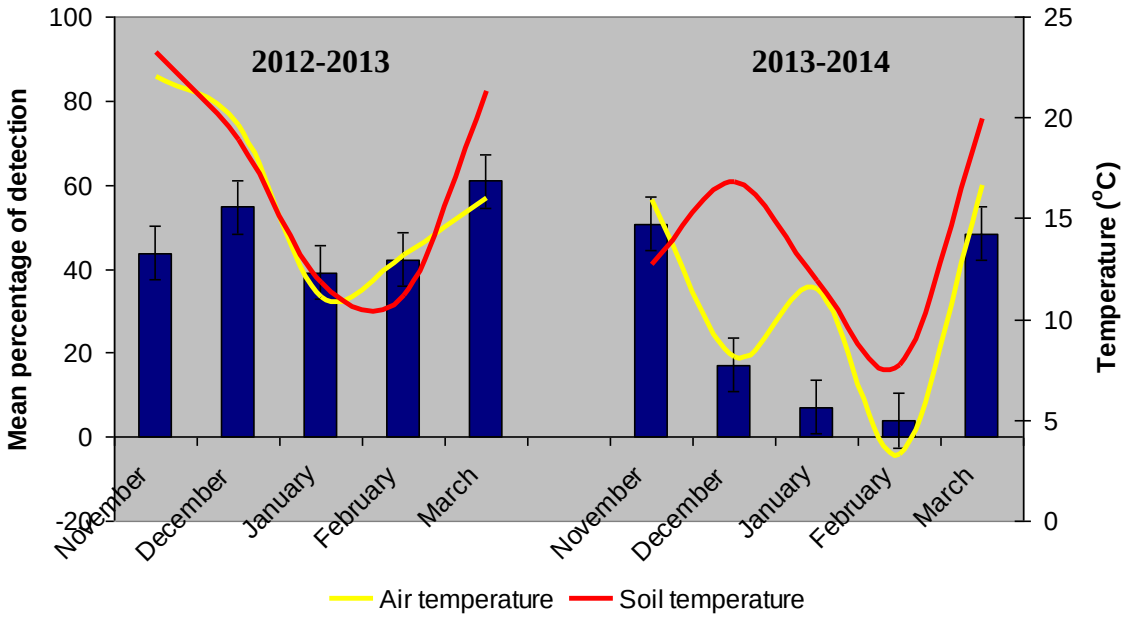
2012-2013

Month	Sampling Spots	Mean % Detection	Mean daily air temperature (ºC)	Mean thatch/soil temperature (°C)
November	64	43.8	22.0	23.2
December	64	54.7	19.6	18.9
January	64	39.1	11.1	11.8
February	64	42.2	13.2	11.2
March	64	60.9	16.0	21.3

2013-2014

Month	Sampling Spots	Mean % detection	Mean daily air temperature (ºC)	Mean thatch/soil temperature (°C)
November	64	50.8	15.9	12.7
December	64	17.2	8.1	16.8
January	64	7.0	11.5	11.9
February	64	3.9	3.3	7.7
March	64	48.4	16.6	19.9

Figure 3.2. The effects of air temperature and thatch/soil temperature on *Rhizoctonia solani* AG 2-2 LP activity per 48-h sampling period each month from November 2012 to March 2013 and from November 2013 to March 2014 at experimental 'Palisades' zoysiagrass plots at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. The blue bars denote detection. The yellow trend line denotes air temperature. The red trend line denotes thatch/soil temperature.



DISCUSSION

As in previous studies (Green et al. 1993, Obasa 2012, Obasa et al. 2013, Smiley et al. 2005), results of this study indicate that summertime irrigation and nitrogen levels are important factors affecting the quality of zoysiagrass as well as thatch/soil moisture, thatch/soil temperature, *R. solani* AG 2-2 LP activity and LP symptom development. Results of this study also indicate that air temperature and thatch/soil temperature are important environmental factors affecting *R. solani* AG 2-2 LP activity prior to the development of LP. A summary of these results is presented in Figure 3.3. Unlike the quality of turfgrass in non-treated control plots, the quality of turfgrass in plots receiving

some level of irrigation and nitrogen was improved. However, differences in quality was not significant when the turfgrass was irrigated or fertilized at higher (100% or 60% of ETo, and 196 kg ha^{-1} N or 98 kg ha^{-1} N, respectively) versus lower (36% of ETo and 49 kg ha^{-1} N, respectively) levels. The lowest levels of irrigation and nitrogen produced acceptable turfgrass. These results are consistent with previous studies that indicated a low requirement of zoysiagrass for cultural inputs to maintain quality (Fry et al. 2008, NTEP 2007, Wherley et al. 2011). Although LP typically occurs in the fall and spring when turfgrass is entering or exiting winter dormancy, respectively, it can also occur in the summer when weather is generally cool and wet (Emmons 2000, Green et al. 1993, Martinez et al. 2009). Higher irrigation levels (100% and 60% of ETo) during the summer in this study were correlated with wetter, cooler thatch/soil in the following fall and spring when *R. solani* AG 2-2 LP was active. Thus, it is not surprising that these same summertime irrigation levels were correlated with *R. solani* AG 2-2 LP activity and LP symptom development in the later season. Conversely, summertime nitrogen levels had no effect on thatch/soil moisture in the later season. They did have an effect on thatch/soil temperature but only during certain sampling periods for *R. solani* AG 2-2 LP in the following fall and spring. Mean thatch/soil temperature generally was the greatest in plots not receiving nitrogen. This is not surprising, as sparse turfgrass observed in these plots likely resulted in less moisture retention and greater solar heating of thatch/soil. As summertime nitrogen level had little to no effect on thatch/soil moisture and thatch/soil temperature in the later season, it is not surprising that it also had little to no effect on *R. solani* AG 2-2 LP activity and LP symptom development.

The effect of summertime nitrogen level on LP symptom development in the following fall and spring was significant only during one sampling period in the second year (March). The only significant difference between nitrogen levels during that sampling period was between 0 kg ha^{-1} and the three actual applications. The mean percentage of LP symptom development was greatest in plots not receiving nitrogen. Although there were no significant differences between actual applications, severity of disease development was the least in plots that received low (49 kg ha^{-1}) and high (196 kg ha^{-1}) rates of nitrogen. Thatch/soil temperature was correlated with *R. solani* AG 2-2 LP activity in both years, and air temperature was correlated with fungal activity in only the second year. As this study indicates, thatch/soil temperature is influenced by moisture. And since time of day and frequency of data collection may have accounted for some of the difference between thatch/soil and air temperatures for a particular sampling period, more study is needed to determine which environmental factor is the better indicator of *R. solani* AG 2-2 LP activity prior to LP symptom development.

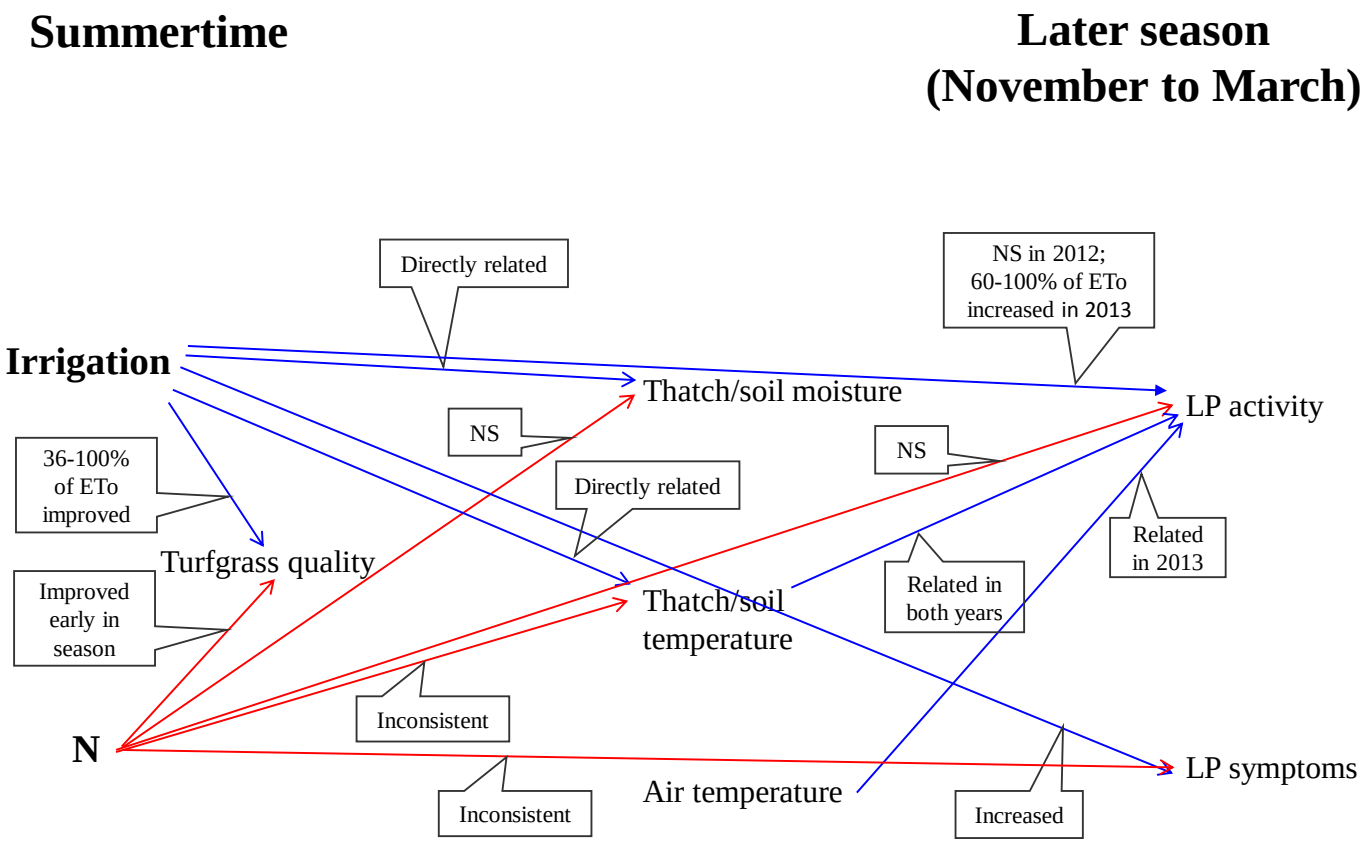
Previous studies have conflicted regarding the importance of nitrogen fertilization in development and severity of disease in general in both cool- and warm-season turfgrasses. Some studies have indicated source, rate and timing of nitrogen application as contributing factors, while others have not (Beard 1982, Bloom and Couch 1960, Braun 2014, Burpee 1995, Cook et al. 1964, Davidson and Goss 1972, Dernoeden 1987, Dernoeden et al. 1991, Dunn et al. 1996, Fidanza and Dernoeden 1996a, 1996b, 1996c; Green et al. 1994, Green et al. 1993, Hill et al. 2001, Kaminski and Dernoeden 2005, Obasa 2012, Obasa et al. 2013, Smiley et al. 1992, Smiley et al.,

2005, Smith 1956, Smith 1958, Thomson et al. 1995, Watkins et al. 1990). The effect of source (urea, urea formaldehyde, poultry litter, sewage sludge and bovine waste) and rate of nitrogen (74 kg ha^{-1} and 148 kg ha^{-1}) applied in the summer on development and severity of LP symptoms the following fall in zoysiagrass were evaluated by Green et al. (1994). The researchers concluded that neither source nor rate had an effect even though their previous study (Green et al. 1993) noted severe disease outbreaks with excessive nitrogen. Since the researchers did not evaluate timing of application, Obasa et al. (2013) conducted a study to determine the effect of nitrogen applied in the fall and spring versus the summer on disease development and severity in zoysiagrass. Urea (46-0-0) was applied once in the summer at a rate of 90 kg ha^{-1}, while polymer-coated urea (41-0-0) was applied once or twice in the fall and spring at the same rate. The researchers concluded that timing of application had no effect. Braun (2014) continued to examine source and timing of nitrogen application and evaluated the effect of ammonium sulfate (21-0-0) and calcium nitrate (15.5-0-0) applied in the fall and spring, and the effect of urea (46-0-0) applied in the summer, on disease development and severity in zoysiagrass. Application at a rate of 73.2 kg ha^{-1} was made in the fall when the soil temperature at a depth of 5 cm was 21°C. Applications at the same rate were made in the spring when the soil temperature at the same depth was 15.5°C and 21°C. Statistical differences between applications occurred on some rating dates. However, Braun (2014) concluded that the differences were not consistent and minimal. The researcher suggested that duration of application, more so than source and timing, may have influenced disease development and severity in zoysiagrass.

In this study, evaluation of the effects of nitrogen fertilization on turfgrass quality, *R. solani* AG 2-2 LP activity and LP symptom development in zoysiagrass included source, rate, timing and duration of application. In contrast to the studies by Green et al. (1994) and Braun (2014), ammonium sulfate (21-0-0) was applied in the summer instead of the fall and spring, and four rates of nitrogen were divided so that they could be applied at six-week intervals throughout the season. Rates included no nitrogen as a control as well as one more excessive than that used in the Green et al. (1994) study. But as in the previous studies, source, rate, timing and duration of nitrogen fertilization in this study had no effect on *R. solani* AG 2-2 LP activity and minimal effect on LP symptom development when applied as a quick-release form within an appropriate application window. It is interesting that greater disease development was observed in plots not fertilized. Conversely, irrigation level had an effect on both fungal activity and disease development but more so on the latter. Temperature, particularly of thatch/soil, had a greater effect on *R. solani* AG 2-2 LP activity. Temperature is speculated to be a trigger for fungal activity in zoysiagrass. Results of this study corroborate published studies and suggest that thatch/soil moisture determines severity of LP symptoms that develop. This is useful information, as environmental conditions may be monitored and used in conjunction with the toothpick baiting method to predict *R. solani* AG 2-2 LP activity and to determine the optimal time to apply fungicides preventatively in the fall. Turfgrass health plays a role in resistance, tolerance or susceptibility to disease. Environmental stressors, such as cold and drought, weaken turfgrass, and can be factors in severity of disease that does develop. Zoysiagrass plots

not fertilized and subjected to environmental stressors for two years in this study may have declined in quality to the point that the turfgrass was more vulnerable to development of LP. This study indicates quality and performance is acceptable and development of LP symptoms is minimized when zoysiagrass is irrigated at 36% to 60% of ETo and fertilized at a rate of 49 kg ha-1. Monitoring of *R. solani* AG 2-2 LP activity using the toothpick baiting method should begin when either the thatch/soil or air temperature is 20°C in the fall and 15°C in the spring.

Figure 3.3. Summary diagram of the key findings in this study. NS = no significance.



CHAPTER IV

RESIDUAL EFFECT OF CHEMICAL CONTROL ON ACTIVITY OF *RHIZOCTONIA SOLANI* AG 2-2 LP AND ON SEVERITY OF LARGE PATCH IN ZOYSIAGRASS

INTRODUCTION

Management of large patch (LP) caused by *Rhizoctonia solani* AG 2-2 LP (Green et al. 1993, Hyakumachi et al. 1998, Smiley et al. 2005) is of particular concern to turfgrass producers and managers, who encounter significant economic loss as a result of chemical inputs to avoid marketing blighted sod, which lowers its value. LP is not curable due to the biology of the causal agent. Once established in the soil, the fungus cannot be eliminated. But disease development may be delayed and/or symptoms suppressed through cultural management of the turfgrass and through fungicide applications in the fall. However, knowledge is still lacking concerning the optimal time to apply fungicides preventatively in zoysiagrass. Thus, treatments often have been based on the calendar and applied in September or October instead of based on changing environmental conditions, such as moisture and temperature. This often results in ineffective and costly applications. A single application is made when nighttime temperatures are ≤ 18°C. But additional applications may be made at 14- to 28-d intervals, depending on product, if environmental conditions conducive to LP development persist into November or December, or if disease develops.

The green industry contributes nearly $15 billion annually to the Texas economy (Palma and Hall 2009), with sod production alone contributing approximately $178 million of that total (Falconer and Niemeyer 2006). Sod ranks as the ninth largest agricultural crop (Falconer and Niemeyer 2006), with production on more than 56,000 acres occurring in the eastern half of the state. Zoysiagrass (*Zoysia* Willd.) is among the most economically important turfgrasses produced and managed in the state. Commercial and residential use is increasing due to the turfgrass' aesthetic appeal (medium to fine texture and rich green color), excellent heat and drought tolerance, good shade and cold tolerance, and low requirement for cultural inputs, such as fertilizer and pesticide (Beard 1972, Chandra et al. 2014, Duble 1989, Fry and Huang 2004, Fry et al. 2008, NTEP 2007, Okeyo et al. 2011, Patton and Reicher 2007, Wherley et al. 2011). But despite its desirable attributes, zoysiagrass is very susceptible to LP (Smiley et al. 2005), and there are no cultivars known to be resistant to the disease. Thus, there is a need for better cultural and chemical management practices, and especially for those practices that would provide an economic benefit to turfgrass producers and managers.

LP often occurs during the fall and spring when turfgrass is entering or exiting winter dormancy, respectively, and when the weather is generally cool and wet (Emmons 2000, Green et al. 1993, Martinez et al. 2009). Disease symptoms in the fall include circular patches of blighted turfgrass, ranging in diameter from less than one meter to up to nearly eight meters (Green et al. 1993, Martinez et al. 2009, Tisserat et al. 1994) (Figure 2.1). Leaf blades at the patch perimeter initially appear yellow, orange or reddish brown in color but then become tan and collapse to the ground, forming a mat

(Green et al. 1993, Merrill 2011). Disease symptoms in the spring include thin, sunken light brown patches in turfgrass that are slower to break dormancy and may be invaded by weeds (Figure 2.1). Symptoms usually disappear and turfgrass recovers in the spring as the soil temperature increases. However, patches of diseased turfgrass tend to return and expand in the same location in the fall when environmental conditions are more favorable.

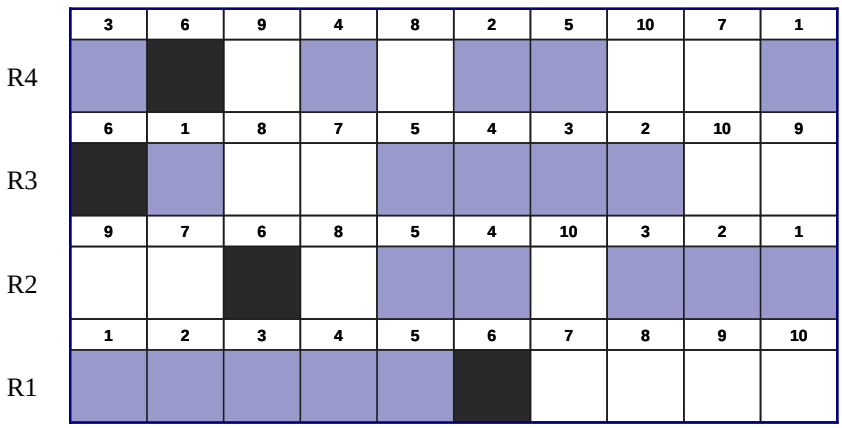
Knowledge is still lacking concerning the optimal time to apply fungicides in zoysiagrass in Texas to delay disease development and/or suppress symptoms. Thus, the objective of this study was to determine the residual effect of chemical control on *R. solani* AG 2-2 LP activity and severity of LP development in zoysiagrass. Data were subjected to statistical analyses to determine relationships.

MATERIALS AND METHODS

Detection of *R. solani* AG 2-2 LP. A toothpick baiting method previously described in a driving range study in Chapter II was used to detect and monitor activity of natural populations of *R. solani* AG 2-2 LP in relation to fungicide treatments for LP at experimental plots established during the fall in 2011, 2012 and 2013 at a driving range at The Club at Carlton Woods in The Woodlands, TX. The driving range is planted in Zeon® Zoysia [*Z. matrella* (L.) Merr.] (Bladerunner Farms, Inc., Poteet, TX) and was not treated for LP. Monthly sampling commenced on the same day or within a few weeks after fungicide applications and continued until green-up of the turfgrass the following spring.

Design and treatments of experimental plots. *2011-2012*. Forty plots (1.2 m x 1.5 m) were set up October 25, 2011, and arranged in a randomized complete block design, with 24 of the plots used for four replications of six treatments (Figure 4.1). Plots were treated the same day and November 15, 2011, with either Chipco 26 GT® (Bayer Environmental Science, Research Triangle Park, NC), Daconil ULTREX® Turf Care® (Syngenta Crop Protection, LLC, Greensboro, NC), Chipco Triton® FLO (Bayer Environmental Science, Research Triangle Park, NC), ProStar® 70WP (Bayer Environmental Science, Research Triangle Park, NC) or Heritage® WG (Syngenta Crop Protection, LLC, Greensboro, NC) (Table 4.1). Each set of replicates included one control plot that was treated with water. Treatments were applied at a pressure of 275.8 kPa and in a spray volume of 81.5 ml m^{-2}, using a CO_2-pressurized boom sprayer equipped with two TeeJet 8002 VisiFlo nozzles (TeeJet Technologies Illinois, LLC, Springfield, IL).

Figure 4.1. Design of 1.2x1.5-meter experimental plots treated with fungicides with topical modes of action in the fall 2011. Treatments (1-6) and their locations are listed to the right and shown in the plot map. Treatment replications (R1-4) are listed to the left.



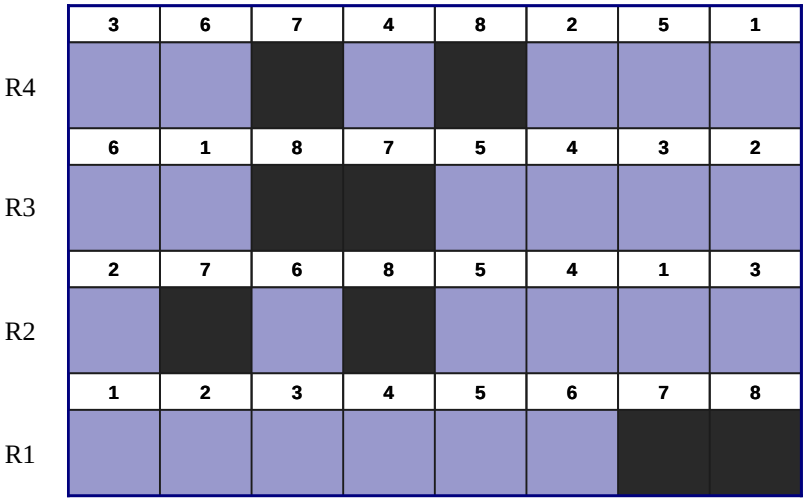
1. Chipco 26 GT®
2. Daconil ULTREX®
3. Chipco Triton® FLO
4. ProStar® 70WP
5. Heritage® WG
6. Control

Table 4.1. Fungicides used in this study are listed with their modes of action, chemical classes, active ingredients and application rates. Fungicides were applied at a pressure of 275.8 kPa and in a spray volume of 81.5 ml m^{-2}, using a CO_2-pressurized boom sprayer equipped with two TeeJet 8002 VisiFlo nozzles (TeeJet Technologies Illinois, LLC, Springfield, IL).

Brand name	Manufacturer	Mode	Chemical class	Active ingredient	Application rate
Chipco 26 GT®	Bayer	localized penetrant	dicarboximide	Iprodione	1.27 ml m^{-2}
Chipco Triton® FLO	Bayer	acropetal	demethylation inhibitor	Triticonazole	0.24 ml m^{-2}
Daconil ULTREX® Turf Care®	Syngenta	contact	nitrile	Chlorothalonil	0.98 g m^{-2}
Heritage® TL	Syngenta	acropetal	strobilurin	Azoxystrobin	0.64 ml m^{-2}
Heritage® WG	Syngenta	acropetal	strobilurin	Azoxystrobin	0.61 g m^{-2}
ProStar® 70WP	Bayer	acropetal	carboximide	Flutolanil	0.67 g m^{-2}
Quali-Pro® ENCLAVE™	Control Solutions	acropetal	nitrile, dicarboximide, triazole, benzimidazole,	chlorothalonil, iprodione, tebuconazole, thiophanate methyl	1.27 ml m^{-2}

2012-2013. Thirty-two plots (1.2 m x1.8 m) were set up September 4, 2012, at a different location on the driving range than in the previous year. Plots were arranged in a randomized complete block design, with four replications of eight treatments (Figure 4.2). Plots were treated September 25, 2012, and November 13, 2012, with the same fungicides as the previous year and with the addition of Quali-Pro® ENCLAVE™ (Control Solutions, Inc., Pasadena, TX) (Table 4.1). A different formulation of Heritage® (TL) also was used (Table 4.1). Each set of replicates included two control plots that were treated with water. Treatments were applied as previously described.

Figure 4.2. Design of 1.2x1.8-meter experimental plots treated with fungicides with topical modes of action in the fall 2012. Treatments (1-8) and their locations are listed to the right and shown in the plot map. Treatment replications (R1-4) are listed to the left.

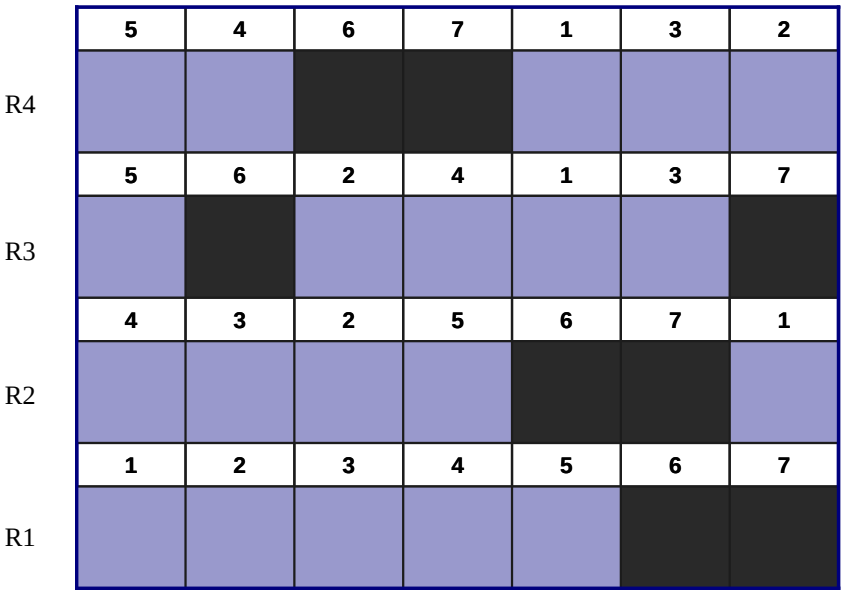


1. Chipco 26 GT®
2. Daconil ULTREX®
3. Chipco Triton® FLO
4. ProStar® 70WP
5. Heritage® TL
6. Quali-Pro® ENCLAVE
7. Control
8. Control

2013-2014. Twenty-eight plots the same dimensions as the previous year were set up September 24, 2013, at a different location on the driving range than in the previous two years. Plots were arranged in a randomized complete block design, with four replications of seven treatments (Figure 4.3). Plots were treated October 9, 2013,

with the same fungicides as the previous year, with the exception of Quali-Pro® ENCLAVE™ (Table 4.1). Each set of replicates included two control plots that were treated with water. Treatments were applied as previously described.

Figure 4.3. Design of 1.2x1.8-meter experimental plots treated with fungicides with topical modes of action in the fall 2013. Design of experimental plots treated with fungicides in the fall 2011. Treatments (1-7) and their locations are listed to the right and shown in the plot map. Treatment replications (R1-4) are listed to the left.



1. Chipco 26 GT®
2. Daconil ULTREX®
3. Chipco Triton® FLO
4. ProStar® 70WP
5. Heritage® TL
6. Control
7. Control

Assessment of treatments and statistical analyses. Treatment effects were evaluated based on the mean number of *R. solani* AG 2-2 LP hyphae that attached to toothpicks per replicated sampling spot and visual assessment of the percentage of area of each experimental plot that exhibited LP symptoms. To determine the residual effect of treatments, data for sampling periods from November to March, when the fungus was speculated to be active, were subjected to Analysis of Variance (ANOVA) using the PROC GLM procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC). Sampling

periods in the three study years were analyzed individually. SAS code used in the analyses is shown in Appendix H. Means of significant independent variables were separated using Fisher's protected least significant difference (LSD) test at $P = 0.05$.

RESULTS

Mean number of hyphae. The effect of treatments on the mean number of *R. solani* AG 2-2 LP hyphae attached to toothpicks was not significant during any sampling period in the first or third years and was only significant in March in the second year (Tables 4.2, 4.3 and 4.4). Comparing the performance of fungicides to control fungal activity during that sampling period, the only significant difference in addition to the control was between Chipco 26 GT® and the other five fungicides (Table 4.5). As expected, the mean number of fungal hyphae was greatest in control plots, followed by Chipco 26 GT®. There were no significant differences between the performance of Daconil ULTREX® Turf Care®, Chipco Triton® FLO, ProStar® 70WP, Heritage® TL or Quali-Pro® ENCLAVE™.

LP symptom development. The effect of treatments on the mean percentage of LP symptom development was significant during all sampling periods in the first year and was only significant in February in the second year (Tables 4.6 and 4.7). LP symptom development in relation to treatments in the third year was not significant during any sampling period (Table 4.8).

Table 4.2. The residual effect of fungicide treatments on *Rhizoctonia solani* AG 2-2 LP activity in Zeon® zoysiagrass from November 2011 to March 2012 at The Club at Carlton Woods in The Woodlands, TX. ND = no detection of hyphae.

		Mean number hyphae attached to toothpicks				
		November†	December	January‡	February§	March¶
SOV	Df	F value	F value	F value	F value	F value
Fungicide	5	2.1	ND	0.66	2.35	0.90

†$P = 0.1191$; ‡$P = 0.6568$; §$P = 0.0915$; ¶$P = 0.5066$

Table 4.3. The residual effect of fungicide treatments on *Rhizoctonia solani* AG 2-2 LP activity in Zeon® zoysiagrass from November 2013 to March 2014 at The Club at Carlton Woods in The Woodlands, TX. ND = no detection of hyphae.

		Mean number hyphae attached to toothpicks				
		November†	December‡	January	February§	March¶
SOV	Df	F value	F value	F value	F value	F value
Fungicide	5	1.49	0.57	ND	1.00	1.64

†$P = 0.2501$; ‡$P = 0.7224$; §$P = 0.4509$; ¶$P = 0.2090$

Table 4.4. The residual effect of fungicide treatments on *Rhizoctonia solani* AG 2-2 LP activity in Zeon® zoysiagrass from November 2012 to March 2013 at The Club at Carlton Woods in The Woodlands, TX.

		Mean number hyphae attached to toothpicks				
		November†	December‡	January§	February¶	March***
SOV	Df	F value	F value	F value	F value	F value
Fungicide	6	0.52	1.00	2.22	1.28	14.31

†$P = 0.7857$; ‡$P = 0.4552$; §$P = 0.0890$; ¶$P = 0.3136$; ***$P < 0.0001$

Table 4.5. The mean number of *Rhizoctonia solani* AG 2-2 LP hyphae that attached to toothpicks in relation to treatments for large patch in Zeon® zoysiagrass from November 2012 to March 2013 at The Club at Carlton Woods in The Woodlands, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$.

	Mean number hyphae attached to toothpicks				
Treatment	November	December	January	February	March
Chipco 26 GT®	0.5000a	0.0000a	0.7500a	7.875a	27.813b
Daconil ULTREX®	0.2500a	0.2500a	0.5000ab	0.875a	7.063c
Chipco Triton® FLO	0.2500a	0.0000a	0.7500a	0.000a	3.750c
ProStar® 70WP	0.7500a	0.0000a	0.7500a	0.438a	6.438c
Heritage® TL	0.2500a	0.0000a	0.5000ab	0.375a	0.000c
Quali-Pro® ENCLAVE™	0.2500a	0.0000a	0.0000b	0.000a	3.250c
Control	0.5000a	0.2500a	1.0000a	7.875a	50.563a
$LSD_{0.05}$	0.8104	0.3624	0.6347	8.5366	14.514

Table 4.6. The residual effect of fungicide treatments on development of large patch in Zeon® zoysiagrass from November 2011 to March 2012 at The Club at Carlton Woods in The Woodlands, TX. NR = data not recorded.

		Mean percentage large patch symptom development				
		November†	December‡	January	February§	March§
SOV	Df	F value	F value	F value	F value	F value
Fungicide	5	5.0	3.6	NR	7.9	7.9

†$P = 0.0068$; ‡$P = 0.0234$; §$P = 0.0008$

Table 4.7. The residual effect of fungicide treatments on development of large patch in Zeon® zoysiagrass from November 2012 to March 2013 at The Club at Carlton Woods in The Woodlands, TX.

		Mean percentage large patch symptom development				
		November†	December‡	January§	February¶	March#
SOV	Df	F value	F value	F value	F value	F value
Fungicide	6	0.79	1.26	1.93	2.61	2.17

†$P = 0.5899$; ‡$P = 0.3229$; §$P = 0.1299$; ¶$P = 0.0537$; #$P = 0.0945$

Table 4.8. The residual effect of fungicide treatments on development of large patch in Zeon® zoysiagrass from November 2013 to March 2014 at The Club at Carlton Woods in The Woodlands, TX. NS = large patch symptoms were recorded, but no statistical output was generated during analyses.

		Mean percentage large patch symptom development				
		November†	December‡	January	February§	March¶
SOV	Df	F value	F value	F value	F value	F value
Fungicide	5	2.30	1.27	NS	1.00	1.04

†$P = 0.0967$; ‡$P = 0.3273$; §$P = 0.4509$; ¶$P = 0.4320$

Comparing the performance of fungicides to control LP symptom development during the first year, the only significant difference was between Chipco 26 GT® and the other four fungicides (Table 4.9). There was no significant difference in performance between Chipco 26 GT® and the control, although more LP symptom development was observed in plots treated with the fungicide. There also were no significant differences between the performance of Daconil ULTREX® Turf Care®, Chipco Triton® FLO, ProStar® 70WP and Heritage® WG. Comparing the performance of fungicides to control LP symptom development in February the second year, all performed better than the control (Table 4.10), as expected, and Chipco 26 GT® performed the worst while Heritage® TL performed the best. There were no significant differences between the performance of Daconil ULTREX® Turf Care®, Chipco Triton® FLO, ProStar® 70WP or Quali-Pro® ENCLAVE™.

Table 4.9. The mean percentage of symptom development in relation to treatments for large patch in Zeon® zoysiagrass from November 2011 to March 2012 at The Club at Carlton Woods in The Woodlands, TX. NR = data not recorded for the sampling period. Means in the same column with the same letter are not significantly different at $P = 0.05$.

	Mean percentage large patch symptom development				
Treatment	November	December	January	February	March
Chipco 26 GT®	12.50b	7.750b	NR	72.50a	72.50a
Daconil ULTREX®	1.25b	2.750b	NR	21.25b	21.25b
Chipco Triton® FLO	2.50b	2.500b	NR	18.75b	18.75b
ProStar® 70WP	2.50b	8.750b	NR	12.50b	12.50b
Heritage® WG	2.50b	3.750b	NR	11.25b	11.25b
Control	47.50a	25.000a	NR	65.00a	65.00a
$LSD_{0.05}$	24.429	13.483	0	29.666	29.67

Table 4.10. The mean percentage of symptom development in relation to treatments for large patch in Zeon® zoysiagrass from November 2012 to March 2013 at The Club at Carlton Woods in The Woodlands, TX. Means in the same column with the same letter are not significantly different at $P = 0.05$.

	Mean percentage large patch symptom development				
Treatment	November	December	January	February	March
Chipco 26 GT®	11.250a	25.00a	48.75ab	52.50ab	55.00ab
Daconil ULTREX®	11.250a	17.50a	12.50bc	22.50bc	41.25ab
Chipco Triton® FLO	11.250a	11.25a	17.50abc	20.00bc	26.25b
ProStar® 70WP	0.000a	1.25a	16.25abc	16.25bc	13.75b

Table 4.10 Continued

	Mean percentage large patch symptom development				
Treatment	November	December	January	February	March
Heritage® TL	0.000a	0.00a	6.25c	7.50c	18.75b
Quali-Pro® ENCLAVE™	10.000a	13.75a	26.25abc	28.75bc	37.50ab
Control	13.000a	26.25a	53.75a	70.00a	75.00a
$LSD_{0.05}$	18.751	27.492	39.268	40.827	43.276

DISCUSSION

Results of field studies to evaluate the efficacy of fungicides to control LP in zoysiagrass have been inconsistent (Green et al. 1994, Gross et al. 1998, Kammerer and Harmon 2008, Tisserat et al. 1994). However, fungicides chosen for this study have been shown to be the most effective. ProStar® 70WP, Heritage® WG/TL, Chipco Triton® FLO and Quali-Pro® ENCLAVE™ are acropetal penetrants and thus have greater residual activity. Acropetal penetrants have protective and curative activity. They form a protective barrier on and penetrate the plant and move upward in the xylem. Chipco 26 GT® is a localized penetrant and thus has less residual activity. Localized penetrants also have protective and some curative activity. They form a protective barrier on and penetrate the plant but do not move in the vascular system. Daconil ULTREX® Turf Care® is a contact fungicide and thus has neither protective nor curative activity. Contact fungicides remain on the outside of the plant and prevent new infection.

The efficacy of fungicides in this study was assessed by visually rating the percentage of diseased turfgrass in each plot and by counting the number of *R. solani* AG 2-2 LP hyphae that attached to toothpicks inserted into each plot. The effect of treatments on fungal activity and disease development was inconsistent within and between study years. Fungicides varied in their residual activity, but all declined in their effect on fungal activity and disease development within three months of application. The effect of treatments was significant only during certain sampling/disease rating dates. But as expected, Chipco 26 GT® generally performed the worst and had the least residual activity, while ProStar® 70WP, Heritage® WG/TL and Chipco Triton® FLO performed the best. ProStar® 70WP and Heritage® WG/TL also had the greatest residual activity.

The toothpick baiting method detects *R. solani* AG 2-2 LP activity at the time of sampling, and activity is not necessarily coincident with the development of LP symptoms. This may account for some of the inconsistency using this method of assessment. Regardless, *R. solani* AG 2-2 LP activity tended to be greater in more symptomatic plots. Also, two fungicide applications were made about a month apart in the fall of 2011 prior to LP symptom development. In the fall of 2012, one application was made before symptoms developed and another was made about two months later after symptoms developed. In the fall of 2013, only one application was made and before symptoms developed. Thus, timing and frequency of application may also account for some of the inconsistency observed within and between treatment years. Previous studies have shown that efficacy of treatments is reduced if fungicides are applied

curatively after LP symptom development (Kammerer and Harmon 2008, Tisserat et al. 1994).

Results from this study and those described in Chapters II and III indicate that monitoring of *R. solani* AG 2-2 LP activity in turfgrass is possible using toothpicks. However, it is doubtful that the method is 100% efficient in terms of frequency of detection, as attachment of hyphae to toothpicks, as an indicator of fungal presence and activity, is likely dependent on environmental conditions. Perhaps the residual effect of fungicides needs to be re-evaluated in a growth chamber study in order to control variability in detection that is present in the field due to thatch/soil moisture, thatch/soil temperature and air temperature. Nevertheless, these studies demonstrate that the toothpick baiting method, used in conjunction with monitoring of environmental conditions, can be a useful tool to predict *R. solani* AG 2-2 LP activity prior to LP symptom development and to determine the optimal time to apply fungicides preventatively in the fall. The method may also be used along with visual assessment of disease symptoms to determine the residual effect of fungicides labeled for LP.

CHAPTER V

CONCLUSIONS

Despite its desirable characteristics, the suitability of zoysiagrass (*Zoysia* Willd.) for residential and commercial use in the southern United States continues to be limited by its susceptibility to large patch (LP) caused by *Rhizoctonia solani* anastomosis group (AG) 2-2 LP (Green et al. 1993, Hyakumachi et al. 1998, Smiley et al. 2005). Knowledge is still lacking concerning specific environmental conditions that contribute to development and severity of LP in this warm-season turfgrass in Texas. There is no zoysiagrass cultivar known to be resistant to LP. But the application of fungicides may delay and/or suppress development and severity of the disease. However, knowledge also is lacking concerning the optimal time to use chemical control. When to treat often is based on the calendar instead of changing environmental conditions, which often results in ineffective and costly fungicide applications.

Zoysiagrass often is managed using practices that were developed for St. Augustinegrass or bermudagrass. Knowledge is still lacking regarding the influence of cultural inputs on quality and performance of zoysiagrass in Texas. Knowledge is also lacking regarding the influence of cultural inputs, in combination with environmental conditions, on development and severity of LP in this turfgrass in Texas. Cultural mismanagement of zoysiagrass in the summer can result in a decline in quality and performance that may make the turfgrass more susceptible to the disease in the following fall and spring. Management of LP is of particular concern to homeowners, and turfgrass

producers and managers, who encounter economic loss due to blighting or dead turfgrass.

Thus, the objectives of this study were to 1) evaluate the seasonal dynamics of *R. solani* AG 2-2 LP activity in the field and laboratory in relation to changing environmental conditions, including thatch/soil moisture, thatch/soil temperature and air temperature; 2) determine the effects of summertime irrigation and nitrogen levels on thatch/soil moisture, thatch/soil temperature, *R. solani* AG 2-2 LP activity and LP symptom development in the following fall and spring; 3) determine the effect of chemical control on *R. solani* AG 2-2 LP activity and LP symptom development; and 4) develop economically sustainable, best-management practices (BMPs) for zoysiagrass in Texas based on data from these studies.

Proper diagnosis of disease and monitoring of the causal agent is imperative in developing BMPs for zoysiagrass that optimize the quality and performance of the turfgrass and make it less susceptible to disease. But isolation of *Rhizoctonia* spp. from the soil can be difficult due to low inoculum density (Paulitz and Schroeder 2005). Researchers developed methods using plant tissue as bait to facilitate the study of fungal activity (Gilligan et al. 1996, LaMondia 1999, Papavizas and Davey 1962, Paulitz et al. 2003, Sneh et al. 1966). Since *Rhizoctonia* spp. can decompose cellulose (Garrett 1962), researchers also developed a method to bait the fungus using toothpicks (Kumar et al. 1999, Paulitz and Schroeder 2005). The method has been used in a number of crop systems and was modified in this study for use in detecting and monitoring *R. solani* AG 2-2 LP in Zeon® Zoysia [*Z. matrella* (L.) Merr.] (Bladerunner Farms, Inc., Poteet, TX)

and ‘Palisades’ zoysiagrass (*Z. japonica* Steud.) (Texas A&M AgriLife Research, Dallas, TX) in relation to environmental and cultural conditions in the field and laboratory. A semi-selective medium, consisting of water agar amended with chloramphenicol, mefenoxim and propiconazole, also was developed in this study to retard the growth of other soil-dwelling fungi (*Bipolaris*, *Curvularia*, *Fusarium*) and *Oomycetes* (*Phytophthora*, *Pythium*) that may be isolated using toothpicks.

In this study, thatch/soil moisture and air temperature were monitored from October 2011 to September 2013 in conjunction with sampling to detect *R. solani* AG 2-2 LP in Zeon® zoysiagrass at a driving range at The Club at Carlton Woods in The Woodlands, TX. Previous studies have shown thatch/soil moisture and thatch/soil temperature to be important environmental factors affecting development and severity of LP symptoms in zoysiagrass (Green et al. 1993, Obasa 2012, Obasa et al. 2013, Smiley et al. 2005). Therefore, we suspected that thatch/soil moisture also would be a factor affecting *R. solani* AG 2-2 LP activity prior to disease development. We also suspected that air temperature influences thatch/soil temperature and hypothesized that air temperature may be used along with thatch/soil moisture to predict fungal activity prior to disease development.

R. solani AG 2-2 LP activity was assessed based on the absence (0) or presence (1) on toothpicks of hyphae characteristic of the fungus. The effects of thatch/soil moisture and air temperature (independent variables) on seasonal *R. solani* AG 2-2 LP activity (dependent variable) were inferred through regression analysis between dependent and independent variables using the REG procedure in SAS version 9.4 (SAS

Institute Inc., Cary, NC). All data were analyzed with respect to year of the study. As expected, both thatch/soil moisture and air temperature were significant environmental factors affecting *R. solani* AG 2-2 LP activity, particularly when they averaged between 28.3% and 53%, and between 9.2°C and 20.8°C, respectively (Figures 2.6, 2.7 and 2.8) (Tables 2.1 and 2.2). The effect of air temperature on *R. solani* AG 2-2 LP activity was consistent with the known range of thatch/soil temperatures (10°C to 21°C) when LP symptoms occur (Tredway and Burpee 2001). Thus, air temperature may be used to infer thatch/soil temperature and subsequently predict fungal activity prior to disease development.

Obasa et al. (2013) noted the absence of LP symptoms in summer field studies in Kansas when recorded thatch/soil temperatures were between 28°C and 29°C. The researchers suggested that the pathogen may be present in zoysiagrass throughout the year, but that disease only occurs when climatic conditions are favorable. Indeed, this was shown by Aoyagi et al. (1998) and Kobayashi (1980), who isolated *R. solani* AG 2-2 LP from symptomless leaf sheaths. Obasa et al. (2013) also suggested that alteration in gene expression in actively growing zoysiagrass at high temperatures may influence susceptibility to LP. But knowledge is still lacking concerning factors other than environmental, including any host-pathogen interaction, which may be involved in disease suppression in the summer, particularly within zoysiagrass genotypes.

Although thatch/soil moisture and air temperature were both significant environmental factors affecting *R. solani* AG 2-2 LP activity in the driving range study, it became apparent by the fact the driving range is routinely irrigated and by differences

in when LP symptoms developed each fall that air temperature may be more important. However, fluctuation in air temperature in the field created a need to conduct a controlled study in the laboratory to better evaluate the effect of seasonal changes on *R. solani* AG 2-2 LP activity in zoysiagrass. Seasonal temperatures associated with fungal activity in the driving range study were subsequently used as treatments in a growth chamber study. For this study, which began June 18, 2014, and ended November 11, 2014, Zeon® zoysiagrass sod plugs were collected from a field nursery at The Club at Carlton Woods in The Woodlands, TX, and transplanted into standard nursery pots containing professional growing medium that had been inoculated with an isolate of *R. solani* AG 2-2 LP using whole oat kernels (Tisserat et al. 1989). Inoculated and non-inoculated control pots also containing Zeon® zoysiagrass sod plugs were subjected twice to a series of decreasing and then increasing temperatures that simulate changing seasonal conditions from fall (20°C, 15°C, 10°C) through spring (15°C, 20°C, 25°C). *R. solani* AG 2-2 activity in sod plugs and growing medium in each pot was quantified separately based on the mean percentage of area on toothpicks that hyphae attached and the mean density of hyphae. Percentage of area was determined by measuring in centimeters the total area on a toothpick containing hyphae, dividing the total by the approximate length of a toothpick (7 cm) and multiplying by 100. Density was determined by a scale developed in this study, whereby 0 = no hyphae present; 1 = hyphae present and countable; 2 = hyphae overlapping but somewhat countable; and 3 = hyphae overlapping and no longer countable. Data were subjected to Analysis of Variance (ANOVA) using the PROC GLM procedure in SAS version 9.3. Means of

significant independent variables were separated using Fisher's protected least significant difference (LSD) test at $P = 0.05$. Data were analyzed with respect to the first, second and combined series of temperature treatments.

The growth chamber study is the first to demonstrate that controlled temperature treatments can be used to evaluate seasonal *R. solani* AG 2-2 LP activity in Zeon® zoysiagrass sod plugs and surrounding growing medium. During the two consecutive series of temperature treatments, the effect of air temperature on *R. solani* AG 2-2 LP activity was significant in both sod plugs and growing medium in terms of attachment and density of fungal hyphae on toothpicks (Table 2.8). However, the fungus tended to be more active in sod plugs when pots were subjected to fall-like temperatures and more active in growing medium when pots were subjected to spring-like temperatures (Tables 2.4 and 2.5). Data seem to indicate that fungal activity shifts from sod plugs to growing medium and then back to sod plugs in relation to seasonal transitions in temperature, particularly between winter (10°C) and spring (15°C), and between summer (25°C) and fall (20°C). If so, this may be due to a survival mechanism in which *R. solani* AG 2-2 LP seeks more favorable environmental conditions during the colder and hotter months. It is known that the fungus colonizes soil in a linear fashion (Kumar et al. 1999). It is feasible that the fungus also colonizes soil vertically in relation to temperature. This was observed somewhat in both the driving range and growth chamber studies in terms of where on toothpicks hyphae attached. Hyphae tended to attach higher on the portion of toothpicks located more in thatch when temperatures were favorable for fungal activity,

and to attach lower on the portion of toothpicks located more in soil when temperatures were above (hotter) or below (colder) those favorable for fungal activity.

The shift in fungal activity between sod plugs and growing medium and vice versa could also indicate the importance of the host in the interaction. It is feasible that changes in gene expression in zoysiagrass entering or exiting dormancy may be sensed by the fungus and trigger movement as well as infection of the host. Both the quality of the sod plugs and activity of *R. solani* AG 2-2 LP in sod plugs and growing medium declined as the study progressed. It is possible that 10g of infested whole oat kernels used to inoculate a pot resulted in diseased sod that failed to recover. It is also possible that a 16-h photoperiod maintained during temperature treatments failed to provide adequate light to compensate for the decrease in temperature that would hinder growth of the sod plugs (Gelernter and Stowell 2005, Glenn 2013, Woods 2013). This would explain the observed decline in sod plugs, but it does not explain the decline in fungal activity in both sod plugs and growing medium. However, it does suggest *R. solani* AG 2-2 LP activity is influenced by growth of the turfgrass, which in turn is influenced by temperature and light. Perhaps day length in addition to temperature is a trigger for changes in gene expression in zoysiagrass entering or exiting dormancy and thus a trigger for increased or decreased pathogen activity as well.

Also in this study, thatch/soil moisture and thatch/soil temperature were monitored from May 2012 to April 2014 in conjunction with an evaluation of the effects of summertime irrigation and nitrogen levels on turfgrass quality, *R. solani* AG 2-2 LP activity and LP symptom development in an established stand of ‘Palisades’ zoysiagrass

at the Texas A&M University Turfgrass Ecology Field Laboratory in College Station, TX. To validate results of the driving range and growth chamber studies, air temperature was also monitored in this study and compared with thatch/soil temperature in relation to influence on *R. solani* AG 2-2 LP activity.

For this study, 64 experimental plots were set up as a split-plot, randomized complete block design with four replications per 16 combinations of treatments (Figure 3.1). Main plot factor was irrigation level, which consisted of a percentage of historical reference evapotranspiration (0, 30, 60 or 100% of ETo) (Texas ET Network, http://texaset.tamu.edu/). Sub plot factor was nitrogen level (0, 49, 98 or 196 kg ha^{-1}). Irrigation was applied three times a week (Tuesday, Thursday and Saturday) during each of two growing seasons. Nitrogen in the form of quick-release ammonium sulfate (21-0-0) was divided into three applications and applied using a drop spreader at six-week intervals beginning in May.

Zoysiagrass in plots was visually rated for turfgrass quality, using a scale of 1 to 9 developed by the National Turfgrass Evaluation Program that was based on color, density, uniformity, texture, disease and environmental stress, whereby 1 = poorest or brown and dead; 5 = minimally acceptable; and 9 = perfect or ideal (http://www.ntep.org/pdf/ratings.pdf). Ratings were conducted on June 1, June 22, July 6, July 24, August 5 and October 10 in 2012, and on June 11, June 28, August 8, August 22 and October 2 in 2013. Plots were assessed for *R. solani* AG 2-2 LP activity based on the absence (0) or presence (1) on toothpicks of hyphae characteristic of the fungus.

Plots were assessed for LP development based on the percentage of area that exhibited symptoms.

To evaluate the effects of summertime irrigation and nitrogen levels, turfgrass quality scores for each rating period, and thatch/soil moisture, thatch/soil temperature, *R. solani* AG 2-2 LP activity and LP symptom development data for the sampling periods from November to March, when the fungus was speculated to be active, were subjected to ANOVA using the PROC GLM procedure in SAS version 9.3. Means of significant independent variables were separated using the LSD test at $P = 0.05$. To compare the influence of thatch/soil temperature and air temperature (independent variables) on *R. solani* AG 2-2 LP activity (dependent variable), data for the sampling periods from November to March were subjected to regression analysis between dependent and independent variables using the REG procedure in SAS version 9.4. Turfgrass quality rating periods were analyzed together with respect to year. Thatch/soil moisture, thatch/soil temperature, *R. solani* AG 2-2 LP activity and LP symptom development data were analyzed together with respect to sampling period. Thatch/soil temperature and air temperature data were analyzed together with respect to year.

The effect of summertime irrigation level on the quality of ‘Palisades’ zoysiagrass was significant during all rating dates in both years, whereas the effect of summertime nitrogen level was significant during three of the six rating dates in the first year and none of the five rating dates in the second year (Table 3.1). Acceptable turfgrass was achieved with an irrigation level of 100% of ETo during all rating dates in both years (Table 3.2). An irrigation level of 60% of ETo also produced acceptable

turfgrass during all rating dates in the first year and during four of the five rating dates in the second year. An irrigation level of 36% of ETo produced acceptable turfgrass during five of the six rating dates in the first year and during one of the five rating dates in the second year. There were no significant differences in turfgrass quality between the 100%, 60% and 36% of ETo irrigation levels in the first year or between the 100% and 60% of ETo irrigation levels in the second year. Acceptable turfgrass was achieved with all nitrogen levels during four of the six rating dates in the first year and during one of the five rating dates in the second year (Table 3.3). Turfgrass quality was significantly improved with the application of nitrogen, but only early in the growing season. However, there were no significant differences in turfgrass quality between the 196 kg ha^{-1} and 98 kg ha^{-1} nitrogen levels.

The effect of summertime irrigation level on *R. solani* AG 2-2 LP activity in the later season was not significant during any of the five sampling periods in the first year but was significant in November and January in the second year (Table 3.9). There were significant differences between irrigation levels during the two sampling periods, with 100% of ETo resulting in the greatest fungal activity (Table 3.10). The effect of nitrogen level on *R. solani* AG 2-2 LP activity in the later season was not significant during any sampling period in either year (Table 3.9).

The effect of summertime irrigation level on LP symptom development in the later season was significant during two (November and January) of the five sampling periods in the first year and during three (November, December and March) of the sampling periods in the second year (Table 3.11). An irrigation level of 60% of ETo in

the first year and of 60% of ETo and 100% of ETo in the second year resulted in the greatest mean percentage of LP symptom development (Table 3.12). The effect of summertime nitrogen level on LP symptom development in the later season was not significant except for one (March) of the sampling periods in the second year when the applications reduced disease symptoms compared to the non-treated control (Tables 3.11 and 3.13).

In both years of this study, higher irrigation levels (100% and 60% of ETo) in the summer generally correlated with wetter, cooler thatch/soil in the later season (Tables 3.4, 3.5, 3.6 and 3.7). Summertime nitrogen level did not affect thatch/soil moisture in the later season (Table 3.4). The effect of summertime nitrogen level on thatch/soil temperature in the later season was not significant except for a few sampling periods (Table 3.6). There were significant differences between thatch/soil temperature caused by nitrogen levels, but there was no consistent trend (Table 3.8).

R. solani AG 2-2 LP activity in the later season in relation to thatch/soil temperature and air temperature was similar to fungal activity observed in relation to only air temperature in the driving range and growth chamber studies. Thatch/soil temperature correlated with *R. solani* AG 2-2 LP activity in both years of this study, while air temperature correlated with fungal activity in only the second year (Figure 3.2) (Table 3.14). Fungal activity tended to increase during the fall, recede during the winter, and again increase in the spring with a seasonal change in air and thatch/soil temperatures. Fungal activity again decreased and eventually became undetectable by late spring once temperatures increased beyond 20°C. *R. solani* AG 2-2 LP activity was

observed when the thatch/soil temperature averaged between 7.7°C and 23.2°C, and when the air temperature averaged between 3.3°C and 22.0°C. The greatest activity in the fall was observed when the thatch/soil temperature averaged between 12.7°C and 18.9°C, and when the air temperature averaged between 15.9°C and 19.6°C. The greatest activity in the spring was observed when the thatch/soil temperature averaged between 19.9°C and 21.3°C, and when the air temperature averaged between 16.0°C and 16.6°C. As this study indicates, thatch/soil temperature is influenced by moisture. And since time of day and frequency of data collection may have accounted for some of the difference between thatch/soil and air temperatures for a particular sampling period, more study is needed to determine which environmental factor is the better indicator of *R. solani* AG 2-2 LP activity prior to LP symptom development.

Previous studies have conflicted regarding the importance of nitrogen fertilization in development and severity of disease in general in both cool- and warm-season turfgrasses. Some studies have indicated source, timing and rate of nitrogen application as contributing factors, while others have not (Beard 1982, Bloom and Couch 1960, Braun 2014, Burpee 1995, Cook et al. 1964, Davidson and Goss 1972, Dernoeden 1987, Dernoeden et al. 1991, Dunn et al. 1996, Fidanza and Dernoeden 1996a, 1996b, 1996c; Green et al. 1994, Green et al. 1993, Hill et al. 2001, Kaminski and Dernoeden 2005, Obasa 2012, Obasa et al. 2013, Smiley et al. 1992, Smiley et al., 2005, Smith 1956, Smith 1958, Thomson et al. 1995, Watkins et al. 1990). Results of the present study were fairly consistent between years, although the effects of irrigation and nitrogen levels on turfgrass quality, *R. solani* AG 2-2 LP activity and LP symptom development were

significant only during certain rating/sampling periods in each year. It is interesting that nitrogen level had no effect on fungal activity and minimal effect on disease development when applied as a quick-release form within an appropriate application window. It is more interesting that greater disease development was observed in plots not fertilized. Conversely, irrigation level had an effect on both fungal activity and disease development but more so on the latter. Temperature, particularly of thatch/soil, had a greater effect on fungal activity. Results of the growth chamber study described in Chapter II suggest *R. solani* AG 2-2 LP activity is influenced by the growth of the turfgrass, which in turn is influenced by light and temperature. Turfgrass health plays a role in resistance, tolerance or susceptibility to disease. Environmental stressors, such as cold and drought, weaken turfgrass, and can be factors in severity of disease that does develop. Zoysiagrass plots not fertilized and subjected to environmental stressors for two years in the present study (Tables 3.24 and 3.25) may have declined in quality to the point that the turfgrass was more vulnerable to development of LP.

Also in this study, the residual effect of fungicides on *R. solani* AG 2-2 LP activity and LP symptom development was evaluated in Zeon® zoysiagrass at the driving range at The Club at Carlton Woods in The Woodlands, TX, during three disease seasons beginning in the fall in 2011. In the first year of the study, 40 plots were set up October 25, 2011, and arranged in a randomized complete block design. Twenty-four of the plots were used for four replications of six treatments (Figure 4.1). Plots were treated the same day and November 15, 2011, with either Chipco 26 GT® (Bayer Environmental Science, Research Triangle Park, NC), Daconil ULTREX® Turf Care® (Syngenta Crop

Protection, LLC, Greensboro, NC), Chipco Triton® FLO (Bayer Environmental Science, Research Triangle Park, NC), ProStar® 70WP (Bayer Environmental Science, Research Triangle Park, NC) or Heritage® WG (Syngenta Crop Protection, LLC, Greensboro, NC). Each set of replicates included one control plot that was treated with water. In the second year, 32 plots were set up September 4, 2012, at a different location on the driving range than in the previous year. Plots were arranged in a randomized complete block design, with four replications of eight treatments (Figure 4.2). Plots were treated September 25, 2012, and November 13, 2012, with the same fungicides as the previous year and with the addition of Quali-Pro® ENCLAVE™ (Control Solutions, Inc., Pasadena, TX). A different formulation of Heritage® (TL) also was used. Each set of replicates included two control plots that were treated with water. In the third year, 28 plots were set up September 24, 2013, at a different location on the driving range than in the previous two years. Plots were arranged in a randomized complete block design, with four replications of seven treatments (Figure 4.3). Plots were treated October 9, 2013, with the same fungicides as the previous year, with the exception of Quali-Pro® ENCLAVE™. Each set of replicates included two control plots that were treated with water.

Results of field studies to evaluate the efficacy of fungicides to control LP in zoysiagrass have been inconsistent (Green et al. 1994, Gross et al. 1998, Kammerer and Harmon 2008, Tisserat et al. 1994). However, fungicides chosen for this study have been shown to be the most effective. ProStar® 70WP, Heritage® WG/TL, Chipco Triton® FLO and Quali-Pro® ENCLAVE™ are acropetal penetrants and thus have greater residual

activity. Acropetal penetrants have protective and curative activity. They form a protective barrier on and penetrate the plant and move upward in the xylem. Chipco 26 GT® is a localized penetrant and thus has less residual activity. Localized penetrants also have protective and some curative activity. They form a protective barrier on and penetrate the plant but do not move in the vascular system. Daconil ULTREX® Turf Care® is a contact fungicide and thus has neither protective nor curative activity. Contact fungicides remain on the outside of the plant and prevent new infection.

The residual effect of fungicides was assessed based on the mean number of *R. solani* AG 2-2 LP hyphae that attached to toothpicks and the mean percentage of area in plots that developed LP symptoms. Data for the sampling periods from November to March, when the fungus was speculated to be active, were subjected to ANOVA using the PROC GLM procedure in SAS version 9.3. Means of significant independent variables were separated using the LSD test at $P = 0.05$. Data were analyzed together with respect to sampling period. The effect of treatments on the mean number of *R. solani* AG 2-2 LP hyphae that attached to toothpicks was not significant during any sampling period in the first or third years and was only significant in March in the second year (Tables 4.2, 4.3 and 4.4). The effect of treatments on the mean percentage of area in plots that developed symptoms was significant during all sampling periods in the first year and only in February in the second year (Tables 4.6 and 4.7). LP symptom development in relation to treatments in the third year was not significant during any sampling period (Table 4.8). The effect of treatments on fungal activity and disease development was inconsistent within and between study years. Treatments varied in

their residual activity, but all declined in their effect on fungal activity and disease development within three months of application. The effect of treatments was significant only during certain sampling/disease rating dates. But as expected, Chipco 26 GT® generally performed the worst and had the least residual activity, while ProStar® 70WP, Heritage® WG/TL and Chipco Triton® FLO performed the best (Tables 4.5, 4.9 and 4.10). ProStar® 70WP and Heritage® WG/TL also had the greatest residual activity.

The toothpick baiting method detects *R. solani* AG 2-2 LP activity at the time of sampling, and activity is not necessarily coincident with the development of LP symptoms. This may account for some of the inconsistency using this method of assessment. Regardless, *R. solani* AG 2-2 LP activity tended to be greater in more symptomatic plots. Also, two fungicide applications were made about a month apart in the fall of 2011 prior to LP symptom development. In the fall of 2012, one application was made before symptoms developed and another was made about two months later after symptoms developed. In the fall of 2013, only one application was made and before symptoms developed. Thus, timing and frequency of application may also account for some of the inconsistency observed within and between treatment years. Previous studies have shown that efficacy of treatments is reduced if fungicides are applied curatively after LP symptom development (Kammerer and Harmon 2008, Tisserat et al. 1994).

Results from this study and those described in Chapters II and III indicate that monitoring of *R. solani* AG 2-2 LP activity in turfgrass is possible using toothpicks. However, it is doubtful that the method is 100% efficient in terms of frequency of

detection, as attachment of hyphae to toothpicks, as an indicator of fungal presence and activity, is likely dependent on environmental conditions. Perhaps the residual effect of fungicides needs to be re-evaluated in a growth chamber study in order to control variability in detection that is present in the field due to thatch/soil moisture, thatch/soil temperature and air temperature. Nevertheless, these studies demonstrate that the toothpick baiting method, used in conjunction with monitoring of environmental conditions, can be a useful tool to predict *R. solani* AG 2-2 LP activity prior to LP symptom development and to determine the optimal time to apply fungicides preventatively in the fall. The method may also be used along with visual assessment of disease symptoms to determine the residual effect of fungicides labeled for LP. *R. solani* AG 2-2 LP activity and development of LP symptoms in relation to nitrogen fertilization is still not clear and may also need to be re-evaluated in a growth chamber study. These studies also suggest that temperature is the trigger for *R. solani* AG 2-2 LP activity prior to the development of LP symptoms, while moisture determines severity of disease. Possible vertical in addition to horizontal movement of the fungus in turfgrass due to changing environmental conditions needs to be evaluated to develop better disease treatment strategies. Turfgrass health also seems to be important for *R. solani* AG 2-2 LP activity and development of LP symptoms. Transcriptome and/or protein analysis is needed to elucidate the nature of these interactions. Such information would be useful in developing zoysiagrass cultivars resistant to LP. But in the meantime, turfgrass producers and managers must rely on cultural and chemical management practices that optimize quality and performance of turfgrass without making it susceptible to LP.

These studies indicate quality and performance is acceptable and development of LP symptoms is minimized when zoysiagrass is irrigated at 36% to 60% of ETo and fertilized at a rate of 49 kg ha^{-1}. Monitoring of *R. solani* AG 2-2 LP activity using the toothpick baiting method should begin when either the thatch/soil or air temperature is 20°C in the fall and 15°C in the spring. Depending on history of LP and level of fungal activity (detection $\geq$ 40% using the toothpick baiting method), the turfgrass may be treated prior to symptom development with a fungicide that is an acropetal penetrant, such as ProStar® 70WP, Heritage® WG/TL or Chipco Triton® FLO. Two applications should be made according to that recommended by the product manufacturer.

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APPENDIX A

SEMI-SELECTIVE MEDIUM FOR ISOLATION OF *RHIZOCTONIA* SPP. FROM TOOTHPICKS INSERTED INTO TURFGRASS

For 500 ml:
7.5g agar (1.5% water agar)
2.083 ml mefenoxim (22.5% active ingredient, 1000 ppm, 1000 mg/L)
1.605 µl propiconazole (1.3 pounds active ingredient per gallon, 0.05 ppm, 5 µg/L)
1.65 ml chloramphenicol (30 mg/ml dissolved in 100% ethanol, 100 mg/L)

1. Prepare water agar and autoclave for 20 min at 121°C.

2. Cool in water bath to 50-60°C.

3. Add mefenoxim, chloramphenicol and propiconazole in that order, and swirl well to mix, as the mefenoxim will precipitate. Note that mefenoxim is extremely viscous, so the pipette tip will need to be rinsed in the medium to ensure the correct amount is added. The minute amount of propiconazole also will require rinsing the pipette tip in the medium.

4. Pour and store plates, bagged, at room temperature.

APPENDIX B

SAS CODE TO DETERMINE THE EFFECT OF THATCH/SOIL MOISTURE AND AIR TEMPERATURE ON *RHIZOCTONIA SOLANI* AG 2-2 LP ACTIVITY IN ZEON® ZOYSIAGRASS AT A DRIVING RANGE AT THE CLUB AT CARLTON WOODS

```
/*1) Carlton Woods golf course data (Year 1)*/
data one;
title 'Carlton Woods golf course data (2011-11 to 2012-03)';
input date moist temp detection;
cards;
;
run;
proc print data=one (obs=10); run;

ods graphics on;
proc reg data=one;
model detection= moist temp;
run;quit;
ods graphics off;
```

APPENDIX C

PROTOCOL FOR TOTAL GENOMIC DNA EXTRACTION

FROM FUNGAL CULTURES

Lysis buffer:
3.152g of 200 mM Tris-HCL (pH 8.0)
1.865g of 50 mM EDTA (pH 5.0)
1.1689g of 200 mM NaCl
1g of 1% n-lauroylsarcosine salt 9 (pH 8.0)

Other chemicals:
70% ethanol (4°C)
100% ethanol (-20°C)
24:1 chloroform:isoamyl alcohol
5 M NaCl
RNase A (10 mg/ml)
TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8.0)
Sterile rubber scrapers or inoculation loops

1. *Rhizoctonia solani* AG 2-2 LP isolate grown 7-10 d on potato dextrose agar

2. Prepare lysis buffer fresh in a screw-top disposable plastic tube.

3. Pipette 1.2 ml of lysis buffer onto culture and scrape mycelium into the buffer using a rubber scraper or inoculation loop. Pipette buffer and mycelium into a 1.5-ml microcentrifuge tube (~600 µl).

4. Incubate tube in a dry bath for 90 min at 65°C.

5. Spin tube for 5 min at 10,000 rpm and then pipette 500 µl into a new 1.5-ml microcentrifuge tube.

6. Add 600 µl 24:1 chloroform:isoamyl alcohol, close tube and invert 10 times.

7. Centrifuge tube 15 min at 8,000 rpm.

8. Transfer supernatant (~300 µl) with a pipette to a new 1.5-ml tube. Be careful not to transfer the white protein layer.

9. Add 5 µl of RNase A and incubate in a dry bath 30 min at 37°C.

10. Add 150 µl of 5 M NaCl (or approximately half the volume in the tube), close tube and invert 15 times.

11. Add 600 µl of 100% ethanol (or approximately twice the volume in the tube), close tube and invert 15 times. A white precipitate may or may not form.

12. Let tube rest 15 min at 4°C.

13. Centrifuge tube for 3 min at 3,000 rpm and then another 3 min at 6,000 rpm to pellet DNA. If pellet is not visible, centrifuge another 3 min at 6,000 rpm. Pellet may or may not be visible at that point but it may still be there.

14. Discard supernatant by pipetting and add 500 µl of 70% ethanol, close tube and invert 10 times.

15. Centrifuge tube for 3 min at 3,000 rpm, discard supernatant by pipetting, centrifuge tube again for 1 min at 3,000 rpm, and discard remaining supernatant by pipetting.

16. Leave tube open to evaporate any remaining ethanol (time will vary). Pellet will turn from opaque to nearly transparent.

17. Resuspend DNA in 50-100 µl of 10 mM TE buffer, pH 8.0, and store tube overnight at 4°C.

18. Long-term store at -20°C.

APPENDIX D

SAS CODE TO DETERMINE THE EFFECT OF TEMPERATURE ON ATTACHMENT AND DENSITY OF *RHIZOCTONIA SOLANI* AG 2-2 LP HYPHAE ON TOOTHPICKS IN A GROWTH CHAMBER STUDY

```
data one;
title 'Growth chamber series 1';
input rep temp$ soil_area soil_density turf_area turf_density;
cards;
;
run;
proc print data=one (obs=10);
run;


/*1)Difference between temperature treatments*/
proc glm data=one;
class rep temp;
model soil_area soil_density turf_area turf_density= temp rep;
means temp/lsd lines;
run;

/*2)Difference in fungal activity between turfgrass area and soil
area*/
ods graphics on;
proc ttest data=one;
paired soil_area*turf_area;
run;

ods graphics off;
/*3)Difference in fungal activity between turfgrass density and soil
density*/
ods graphics on;
proc ttest data=one;
paired soil_density*turf_density;
run;
ods graphics off;
```

APPENDIX E

SAS CODE TO DETERMINE THE EFFECTS OF PRIOR SUMMERTIME IRRIGATION AND NITROGEN LEVELS ON THE QUALITY OF 'PALISADES' ZOYSIAGRASS AT THE TEXAS A&M UNIVERSITY TURFGRASS ECOLOGY FIELD LABORATORY

```
data TAMU2012;
title "Turf quality year 1";
input rep irrg nitro qua1 qua2 qua3 qua4 qua5 qua6;
cards;
;
proc glm;
    class rep irrg nitro;
    model qua1 qua2 qua3 qua4 qua5 qua6= rep irrg rep*irrg nitro
nitro*irrg;
    test h=irrg e=rep*irrg;
    means irrg/LSD ALPHA= 0.05;
    means nitro/LSD ALPHA= 0.05;
      means nitro*irrg;
    run;
```

APPENDIX F

SAS CODE TO DETERMINE THE EFFECTS OF PRIOR SUMMER IRRIGATION AND NITROGEN LEVELS ON THATCH/SOIL MOISTURE AND TEMPERATURE, *RHIZOCTONIA SOLANI* AG 2-2 LP ACTIVITY AND LARGE PATCH SYMPTOM DEVELOPMENT IN THE FOLLOWING FALL AND SPRING IN 'PALISADES' ZOYSIAGRASS AT THE TEXAS A&M UNIVERSITY TURFGRASS ECOLOGY FIELD LABORATORY

```
data January TAMU2013;
title "January Soiltemp Soilmoist Rhizoc Sympt 2013";
input rep irrg nitro soilmst soiltemp rhizoc sympt;
cards;
;
proc glm;
    class rep irrg nitro;
    model soilmst soiltemp rhizoc sympt= rep irrg rep*irrg nitro
nitro*irrg;
    test h=irrg e=rep*irrg;
    means irrg/LSD ALPHA= 0.05;
    means nitro/LSD ALPHA= 0.05;
      means nitro*irrg;
    run;
```

APPENDIX G

SAS CODE TO DETERMINE THE EFFECTS OF AIR TEMPERATURE AND THATCH/SOIL TEMPERATURE ON *RHIZOCTONIA* SPP. ACTIVITY IN 'PALISADES' ZOYSIAGRASS AT THE TEXAS A&M UNIVERSITY TURFGRASS ECOLOGY FIELD LABORATORY

```
/*1) TAMU (Year 1)*/
data one;
title 'TAMU turf plots 2012-2013';
input month air soil detection;
cards;
;
run;
proc print data=one (obs=10); run;

ods graphics on;
proc reg data=one;
model detection= air soil;
run;quit;
ods graphics off;
```

APPENDIX H

SAS CODE TO DETERMINE THE RESIDUAL EFFECT OF CHEMICAL CONTROL

ON ATTACHMENT OF *RHIZOCTONIA SOLANI* AG 2-2 LP HYPHAE

ON TOOTHPICKS AND DEVELOPMENT OF LARGE PATCH SYMPTOMS

IN ZEON® ZOYSIAGRASS AT A DRIVING RANGE

AT THE CLUB AT CARLTON WOODS

```
data Fungicide Trial1;
Title "Fungicide Yr 1 Nov";
input rep trt hyp sev;
cards;
;
proc glm;
class rep trt hyp sev;
model hyp sev= trt rep;
means trt/LSD lines;
run;
```